Issued November 17, 1913.

U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF CHEMISTRY—BULLETIN No. 162.

CARL L. ALSBERG, Chief of Bureau.

PROCEEDINGS

OF THE

TWENTY-NINTH ANNUAL CONVENTION

OF THE

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS,

HELD AT

WASHINGTON, D. C., SEPTEMBER 16–18, 1912.

EDITED BY

W. D. BIGELOW,
SECRETARY OF THE ASSOCIATION,

WITH THE COLLABORATION OF

G. O. SAVAGE,
Editor, Bureau of Chemistry.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1913.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., April 11, 1913.

Sir: I have the honor to submit for your approval the Proceedings of the Twenty-ninth Annual Convention of the Association of Official Agricultural Chemists. All general discussion has been omitted, only the reports and correlated papers which affect the conduct of the work being presented. I recommend that these proceedings be published as Bulletin 162 of the Bureau of Chemistry.

Respectfully,

Carl L. Alsberg,
Chief of Bureau.

Hon. D. F. Houston,
Secretary of Agriculture.

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FIRST DAY.

MONDAY—MORNING SESSION.

The twenty-ninth annual convention of the Association of Official Agricultural Chemists was called to order by the president, Mr. H. J. Patterson, of College Park, Md., on the morning of September 16, at the Raleigh Hotel, Washington, D. C. The following members and visitors were present:

MEMBERS AND VISITORS PRESENT.

Adams, A. B., Bureau of Internal Revenue, Treasury Department, Washington, D. C.
Albright, A. R., Bureau of Chemistry, Washington, D. C.
Almy, L. H., Bureau of Chemistry, Washington, D. C.
Alsberg, C. L., Bureau of Plant Industry, Washington, D. C.
Appleman, C. O., College Park, Md.
Averitt, S. D., Agricultural Experiment Station, Lexington, Ky.

Bacon, C. B., Bureau of Chemistry, Washington, D. C.
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Bailey, H. S., Bureau of Chemistry, Washington, D. C.
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Bidwell, G. L., Bureau of Chemistry, Washington, D. C.
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Cameron, F. K., Bureau of Soils, Washington, D. C.
Carpenter, F. B., Virginia-Carolina Chemical Co., Richmond, Va.
Carter, A. J. C., Bartlett, Hayward Co., Baltimore, Md.

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REPORT ON PHOSPHORIC ACID.

By H. D. Haskins, *Referee*, and A. J. Patten, *Associate Referee*.

The work on phosphoric acid has been along lines recommended by the referee for 1911, namely, further study of the application of the official volumetric method in the determination of both the available and total phosphoric acid in basic slag phosphate, and further study of the citrate of ammonia magnesia mixture method in the determination of available phosphoric acid, using the Wagner method of making the citric solution of the slag. Some delay was experienced in sending samples, as it was the desire of the referee to include the slag which was to be used by the special committee in conducting vegetation experiments. Samples of three different grades of slag were prepared and sent, with instructions, about June 1 to twelve chemists, who, in response to our secretary's circular, had signified their desire to cooperate in this line of the work. The instructions were as follows:

Instructions for Cooperative Work of 1912.

Dear Sir: There are being sent to you by express three samples of basic slag phosphate. Please have them analyzed as per methods outlined below.

A. Determine moisture at 100° C.

B. Total phosphoric acid.

(a) Determine total phosphoric acid in each by the official gravimetric method, using the a_4 method of making solution. (See Bul. 107, Rev., Bureau of Chemistry.)

(b) Determine total phosphoric acid in each by the official gravimetric method, using the a_7 method of making solution.

(1) Dehydrate an aliquot of solutions (a_7) by evaporating to dryness on a steam or hot-water bath; take up with 5 cc of hydrochloric acid and 25 cc of warm water; digest to complete the solution and filter off the silicon dioxid; wash six times with hot water. Determine phosphoric acid in solutions by the official gravimetric method.

(2) Determine phosphoric acid in an aliquot of solutions (a_7) by the optional volumetric method. (See (b), p. 4., Bul. 107, Rev., Bureau of Chemistry.)

C. Determine available phosphoric acid as follows:

(a) *Making citric solution.*—Weigh 5 grams of the basic slag, transfer to a one-half liter Wagner flask containing 5 cc of 95 per cent alcohol. The flask should have a neck width of at least 20 mm and be marked at least 8 centimeters below the mouth. Make up to the mark with dilute citric acid solution (2 per cent) of a temperature of 17.5° C. Fit the flask with a rubber stopper and put at once into the rotary apparatus for 30 minutes, making 30 to 40 revolutions per minute. Take off and filter immediately.

(b) *Analysis of the citric solution.*—As soon as the filtration is completed analyze at once according to the following methods:

(1) *Molybdate method* (provisionally adopted 1911).—To 50 cc of the clear filtrate add 100 cc of molybdate solution made according to the official methods. Put the beaker into a water bath until the temperature reaches 65° C.; take out and allow to cool at ordinary temperature. Then filter, and wash the yellow precipitate of phosphomolybdate of ammonia four or five times with 1 per cent nitric acid. Dissolve in 100 cc of 2 per cent ammonium hydroxid (cold), nearly neutralize with hydrochloric acid, and add to the solution 15 cc of magnesia mixture (made according to the official method) drop by drop during continuous stirring. After 15 minutes add 10 to 12 cc of ammonium hydroxid solution (specific gravity 0.90), then cover the beaker with a glass cover and allow to stand for about 2 hours. Filter the double phosphate of ammonia and magnesia through a tared platinum Gooch crucible, wash six times with 2 per cent ammonium hydroxid, dry, and proceed as customary for phosphoric acid determinations.

(2) *Optional volumetric method.*—Determine phosphoric acid in an aliquot of the clear solutions by the optional volumetric method. (See (b), p. 4, Bul. 107, Rev.)

(3) *Citrate of ammonia magnesia mixture method.*—Place 100 cc of the clear filtrate into a 200 cc flask and add 50 cc of citrate magnesia mixture (made by placing 200 grams of citric acid and 40 grams of ammonium chlorid in a liter flask, adding 200 cc of water and 500 cc of ammonium hydrate (20 per cent), keeping the flask stoppered until the contents are dissolved and cooled down, then adding 55 grams of chlorid of magnesia and filling up to the mark with water). Heat the flask slightly (about 15 minutes) by means of a Bunsen burner turned low, until the silica (SiO_2) has been precipitated. Shake the flask in order to conglomerate the precipitate and continue heating

to the boiling point. Allow to cool, add 25 cc of hydrochloric acid (specific gravity 1.124), and allow it to stand about 30 minutes with occasional shaking. Fill up to the mark with water, insert rubber stopper in flask, and shake vigorously several times until the silica (SiO_2) precipitate has been divided into very fine particles. Filter and to 100 cc of the filtrate (0.5 gram basic slag) add 50 cc of a 10 per cent solution of ammonia while stirring the contents of the beaker. Continue stirring, preferably by means of a stirring apparatus, for 30 minutes, filter the precipitate, and treat as usual.

PREPARATION OF SOLUTIONS.

1. *Concentrated solution of citric acid (10 per cent).*—Dissolve in water exactly 200 grams of chemically pure crystallized citric acid having its full percentage of water of crystallization. Make up to exactly 2 liters. (Where a large number of analyses are to be made, 0.5 gram of salicylic acid should be added to the liter of this solution to prevent decomposition.)

2. *Dilute solution of citric acid (2 per cent).*—Mix exactly 1 volume of the concentrated citric-acid solution with 4 volumes of water. The resulting solution should have a temperature of about 17.5° C. when used.

PRECAUTIONS AND FURTHER INFORMATION.

1. A photograph and detailed drawings of an inexpensive but efficient shaking apparatus were sent out with last year's instructions for phosphoric-acid work. A copy will be forwarded, on request, to anyone cooperating in the work this year.

2. The rotary apparatus prescribed for shaking the flasks must not be substituted by ordinary shaking or rocker apparatus, as the latter differs in construction and effect. The rotary apparatus must turn round its axle 30 to 40 times per minute. Variation within these limits has no marked influence on the results.

3. The half-liter flasks (after the design of Wagner) must have a neck width of at least 20 mm and are marked at least 8 centimeters below the mouth. These two points are important, for if the neck width is too narrow and the mark too high the result will be too low, owing to the movement of the liquid being so limited. (The proper flasks are listed in E. and A. Catalogue, see No. 4589a.)

4. The filtration must be done immediately after 30 minutes' rotation, and it is recommended to use a folded filter paper of such size that the whole quantity of liquid can be poured onto the filter at once. Small and bad filtering papers give rise to error in consequence of too slow filtration. If at first the filtrate is not clear it must be again filtered (through the same filter) until it becomes clear.

5. After filtering the citric solution of the basic slag, the precipitation of the phosphoric acid should be carried along without delay, as long standing increases the tendency of the silica to precipitate during the operation.

6. If the beaker containing the mixture of phosphatic and molybdic solutions is put into the water bath until the temperature reaches between 60° and 70° C., a precipitate free from silicic acid results. If heating is continued for a considerably longer time the precipitate will often be mixed with silicic acid, especially when the molybdic solution is not added to the filtrate immediately, but only after 6 to 12 hours (or longer) after filtration. If silicic acid is present, the precipitate dissolves slowly in ammonium hydroxid, but at first not clearly. Special attention must be paid to the point that the yellow precipitate is dissolved quickly and quite clearly by ammonium hydroxid (2 per cent) not made warm. If the solution becomes clear only after some time, molybdic solution and nitric acid must be added to same in order to get a pure precipitate of phospho-molybdate of ammonia; in other words, the phosphoric acid must be reprecipitated by the molybdic solution.

Results of Collaborative Work.[a]

Table 1.—*Comparative work on basic slag.*

TOTAL PHOSPHORIC ACID.

Chemist.	Sample 1.				Sample 2.				Sample 3.			
	Official gravimetric method.			Optional volumetric method, solution a_7.	Official gravimetric method.			Optional volumetric method, solution a_7.	Official gravimetric method.			Optional volumetric method, solution a_7.
	Solution a_4.	Solution a_7.	Solution a_7 dehydrated.		Solution a_4.	Solution a_7.	Solution a_7 dehydrated.		Solution a_4.	Solution a_7.	Solution a_7 dehydrated.	
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
A. J. Patten,[1] Michigan	19.07	18.97	19.04	18.90	17.98	17.88	18.07	17.75	15.10	15.18	15.37	15.05
P. L. McCreary and P. L. Hibbard,[1] California	19.25	18.55	18.51	18.30	18.27	17.27	17.03	16.70	15.50	14.73	14.66	14.40
L. S. Walker,[2] Massachusetts	18.49	18.32	18.63	18.03	17.11	16.85	16.89	16.57	14.69	14.53	14.68	14.27
J. C. Jurrjens,[3] Wisconsin	18.05	17.64	.....	.....	17.03	16.09	.....	.....	14.38	14.08	.....	.....
Average	18.94	18.61	18.73	18.41	17.79	17.33	17.33	17.01	15.10	14.81	14.90	14.59

[1] Average of two determinations. [2] Average of three determinations. [3] Not included in average.

AVAILABLE PHOSPHORIC ACID.

Chemist.	Sample 1.			Sample 2.			Sample 3.		
	Molybdate method.	Optional volumetric method.	Citrate of ammonia-magnesia mixture method.	Molybdate method.	Optional volumetric method.	Citrate of ammonia-magnesia mixture method.	Molybdate method.	Optional volumetric method.	Citrate of ammonia-magnesia mixture method.
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
A. J. Patten,[1] Michigan	14.97	[2] 14.93	14.87	14.67	[2] 14.65	14.70	12.93	[2] 12.98	12.87
P. L. McCreary and P. L. Hibbard,[1] California	14.89	14.67	14.93	15.01	14.80	15.04	13.10	13.00	13.11
L. S. Walker,[3] Massachusetts	14.75	13.43	15.19	15.01	13.87	15.19	13.00	12.99	13.00
Average	14.87	14.34	15.00	14.90	14.44	14.98	13.01	12.99	12.99

[1] Average of two determinations.
[2] Solution evaporated to dryness, ignited, and residue digested with nitric acid. Low results were obtained except when this modification was followed.
[3] Average of three determinations.

COMMENTS BY ANALYSTS.

A. J. Patten, East Lansing, Mich.: In determining the available phosphoric acid by the optional volumetric method, low results were obtained except when the solution was evaporated to dryness, ignited, and then digested with nitric acid and directions followed.

P. L. McCreary and P. L. Hibbard, Berkeley, Cal.: It was the opinion of these analysts that the method of dehydration in the determination of the total phosphoric acid (solution made according to a_7) is not suited to these samples, and that in all cases in the determination of total phosphoric acid (P_2O_5) by the gravimetric method the precipitate was contaminated with iron and silica compounds.

[a] Complete results have been received from only three chemists.

DISCUSSION OF RESULTS.

In the determination of total phosphoric acid on the three slags the various analysts do not obtain results in all cases showing a close agreement. Of the various methods, the optional volumetric gives the lowest results in all cases. The official gravimetric method gave on the average higher results in cases where sulphuric acid was used as a solvent than where hydrochloric acid and nitric acid were used.

Dehydration of the solution of slag where aqua regia was used as a solvent did not appear to be effective in removing all of the silica from the solution, as results by this method agree fairly well with solution made according to the other method, in which the solution was not dehydrated.

If the magnesia precipitate is contaminated with iron and silica (SiO_2) compounds, as was noted in case of the California analysts, the volumetric method offers a relief from this trouble. The referees are of the opinion, however, that an insufficient amount of work has been done with it as yet on basic slag to warrant the drawing of final conclusions.

In the determination of available phosphoric acid in basic slags, the results of the various analysts on Samples 1 and 2 do not agree as closely as might be desired, while on Sample 3 they agree very closely, and the various methods gave concordant results. The volumetric method, as a rule, has given lower results than the gravimetric methods, while the citrate of ammonia magnesia mixture method has, on the average, given somewhat higher results than either of the other two.

It is to be regretted that only three analysts were able to take part in the phosphoric-acid work this year. This is not attributed to a lack of interest, but to the fact that the samples were sent out rather late and the annual meeting was called nearly two months earlier than usual, which left a much shorter time in which to do the work. A sufficient amount of each grade of slags has been reserved to be used for several years' work. It should be borne in mind that it is not desirable for the association to adopt as official any method for the analysis of basic slag phosphates until the special committee has finished several years' study of this phosphoric acid source by means of vegetation experiments.

It is therefore recommended—

(1) That further work be done next year on the methods for basic slags with the methods and samples used by the referee this year.

The following report was submitted by Mr. B. B. Ross at the request of Mr. Hare:

REPORT ON NITROGEN.

By C. L. HARE, *Referee.*

In accordance with the recommendations made in 1911, the following samples were prepared and 14 sets, together with the instructions outlined below, were sent to chemists who had expressed a willingness to cooperate in the work.

INSTRUCTIONS FOR COOPERATIVE WORK OF 1912 ON NITROGEN.

Sample 1: Cottonseed meal (contains about 6 per cent water-insoluble organic nitrogen).

Sample 2: Treated leather (contains about 5.5 per cent water-insoluble organic nitrogen).

Sample 3: Hide and skin meal (contains about 7.5 per cent water-insoluble organic nitrogen).

Sample 4: Mixed fertilizer made up from acid phosphate, muriate of potash, and portions of Nos. 1, 2, and 3 (contains about 2.7 per cent water-insoluble organic nitrogen).

Sample 5: Chemically pure nitrate of soda.

METHODS TO BE EMPLOYED.

1. A. Determine total nitrogen in each sample by one of the official methods.

B. Determine total nitrogen in Sample 4 by the Gunning method as modified below. Approximately 0.7 grams of mercuric oxid, or its equivalent in metallic mercury, may also be added before the addition of the potassium sulphate, but if mercury be used potassium sulphid must be employed, as in the Kjeldahl method in the distillation.

2. Determine available nitrogen in Samples 1, 2, 3, and 4 by the alkaline and neutral permanganate methods as outlined below:

Alkaline permanganate method.

Activity with mixed fertilizers.—Transfer an amount of material equivalent to 50 mg of water-insoluble organic nitrogen to a filter paper and wash with successive portions of water at room temperature until the filtrate amounts to about 250 cc.

Activity with raw materials.—Transfer an amount of material equivalent to 50 mg of water-insoluble organic nitrogen to a small mortar, add about 2 grams of powdered rock phosphate, mix thoroughly, transfer to a filter paper, and wash with successive portions of water at room temperature until the filtrate amounts to about 250 cc. When much oil or fat is present, it is well to wash with ether before extracting with water.

Dry the residue at a temperature not exceeding 80° C. and transfer from the filter to a 500–600 cc Kjeldahl distillation flask (round bottom preferred, but if flat bottom is used, incline at an angle of 30 degrees). Add 20 cc of water, 15 to 20 small glass beads to prevent bumping, and 100 cc of alkaline permanganate solution (25 grams pure potassium permanganate and 150 grams sodium hydroxid, separately dissolved in water, the solutions cooled, mixed, and made to volume of 1 liter). Connect with an upright condenser to which a receiver containing standard acid has been attached. Digest slowly, below distillation point, with very low flame, using coarse wire gauze and asbestos paper between flask and flame, for at least 30 minutes. Gradually raise the temperature and when danger (if any) from frothing has ceased, distill until 95 cc of distillate is obtained, and titrate as usual. In cases where a tendency to froth is noticed, lengthen the digestion period and no trouble will be experienced when the distillation is begun. During the digestion gently rotate the flask occasionally, particularly if the material shows a tendency to adhere to the sides. It is recommended that as nearly as possible 90 minutes be taken for the digestion and distillation. The nitrogen thus obtained is the active water-insoluble organic nitrogen.

Neutral permanganate method.

Weigh a quantity of the fertilizer equivalent to 50 mg of water-insoluble organic nitrogen on a moistened 11-centimeter filter paper and wash with successive portions of water at room temperature until the filtrates amount to 250 cc. Transfer insoluble residue with 25 cc of tepid water to a 300 cc low-form Griffin beaker, add 1 gram of sodium carbonate, mix, and add 100 cc of 2 per cent permanganate solution. Digest in a steam or hot-water bath for 30 minutes at the temperature of boiling water, covering the beaker with a watch glass and setting well down into the bath so that the level of the liquid in the beaker is below that of the bath. Stir twice at intervals of 10 minutes. At the end of the digestion remove from the bath, add 100 cc of cold water, and filter through a heavy 15-centimeter folded filter. Wash with cold water, small quantities at a time, until total filtrate amounts to about 400 cc. Determine nitrogen in residue and filter, correcting for the nitrogen of the filter.

3. Determine nitrogen in Sample 5 as follows: To 0.5 gram of the nitrates in 600 to 700 cc flask add 200 cc of distilled water, 5 grams of powdered zinc, from 1 to 2 grams of ferrous sulphate, and 50 cc of a 36° Baumé soda solution. In the neck of the flask place some glass wool and connect with the distilling apparatus. Distill off the ammonia and collect as usual in decinormal sulphuric acid and titrate.

Tabulated Results of Collaborative Work.

Table 1.—*Comparative work on nitrogen.*

Chemist.	Sample 1.			Sample 2.			Sample 3.			Sample 4.			Sample 5.	
		Active water-insoluble nitrogen.			Active water-insoluble nitrogen.			Active water-insoluble nitrogen.			Active water-insoluble nitrogen.		Nitrogen.	
	Total nitrogen.	Alkaline permanganate method.	Neutral permanganate method.	Total nitrogen.	Alkaline permanganate method.	Neutral permanganate method.	Total nitrogen.	Alkaline permanganate method.	Neutral permanganate method.	Total nitrogen.	Alkaline permanganate method.	Neutral permanganate method.	Official method.	Proposed method.
	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
L. S. Walker, Amherst, Mass.	6.96	3.94	0.37	6.75	2.84	1.26	8.11	3.94	1.94	3.07	1.52	0.44	.....	15.94
	6.82	4.23	.....	.....	.....	.....	8.05	.....	1.60	3.01	.....	.....	16.22	16.80
J. T. Jurrjens, Madison, Wis.	6.73	4.20	.26	.....	.....	.....	7.80	5.30	1.64	3.07	.....	.41	16.44	16.88
	6.72	4.25	.30	.....	.....	.....	7.80	5.24	1.56	3.00	1.48	.39	.....	.....
	.....	.....	.....	.....	.....	.....	.....	.....	.....	3.06	1.43	.36	.....	.....
E. M. Bailey, New Haven, Conn.	.....	3.38	.38	6.85	2.66	1.52	8.20	4.18	.....	[1]3.10	1.47	.32	16.40	16.42
	7.12	3.33	.49	6.84	2.42	1.58	8.22	4.46	1.38	3.05	1.57	.29	16.40	16.42
C. H. Jones, Burlington, Vt.	6.91	4.08	.27	6.66	2.92	1.08	8.00	4.65	1.21	3.12	1.56	.25	.....	16.00
	6.95	3.99	.23	6.73	2.77	1.04	8.00	4.54	1.54	3.09	1.52	.31	16.42	16.34
	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	16.36	16.20
C. L. Hare and J. B. Jackson, Auburn, Ala.	6.85	2.95	.34	6.54	2.65	1.08	.....	3.71	1.60	.....	.....	.34	.....	16.25
	6.84	3.16	.34	6.54	2.80	1.08	.....	3.71	1.58	2.91	1.33	.53	16.12	16.30
	.....	.....	.....	.....	.....	1.17	.....	.24	1.05	2.91	1.39	.34	.....	.....
	.....	.....	.....	.....	.....	1.17	7.84	3.60	.90	.....	.....	.....	.....	.....
Arao Itano, East Lansing, Mich.	7.00	4.71	.....	6.68	5.13	.....	8.00	5.28	.....	3.00	.....	.47	.....	.....
	6.96	4.53	.....	6.62	5.13	.....	8.10	5.33	.....	2.91	1.80	.54	.....	.....
McCandless Laboratory, Atlanta, Ga.	7.06	.....	.34	6.81	.....	1.24	8.05	.....	1.44	3.09	.....	.30	.....	.....
Average.	6.91	3.89	.32	6.70	3.26	1.22	8.01	4.40	1.45	3.03	1.51	.40	16.34	16.36

[1] Determinations by modified Gunning method gave 3.13 and 3.07 per cent.

Table 2.—*Availability percentages.*[1]

Chemist.	Sample 1.		Sample 2.		Sample 3.		Sample 4.	
	Alkaline potassium permanganate.	Neutral potassium permanganate.	Alkaline potassium permanganate.	Neutral potassium permanganate.	Alkaline potassium permanganate.	Neutral potassium permanganate.	Alkaline potassium permanganate.	Neutral potassium permanganate.
L. S. Walker, Amherst, Mass.	64.4	94.7	55.1	80.1	51.1	76.1	60.0	86.0
J. T. Jurrjens, Madison, Wis.	70.5	95.8	.....	.....	69.4	79.8	57.4	88.8
E. M. Bailey, New Haven, Conn.	54.6	96.4	50.0	77.3	55.2	83.2	60.0	90.0
C. H. Jones, Burlington, Vt.	62.7	96.1	55.5	83.0	60.1	85.1	57.4	89.8
C. L. Hare and J. B. Jackson, Auburn, Ala.	52.5	95.0	55.2	82.9	50.0	80.0	57.0	86.0
Arao Itano, East Lansing, Mich.	66.1	.....	77.2	.....	65.9	.....	61.2	.....
McCandless Laboratory, Atlanta, Ga.	.....	95.2	.....	82.0	.....	81.0	.....	90.0

[1] The availability percentages by the alkaline permanganate method are all calculated on the basis of the following percentages of water-soluble nitrogen: Sample 1, 0.54; Sample 2, 0.88; Sample 3, 0.21; Sample 4, 0.28.

No comments by analysts accompanied any of the results. As moisture determinations were reported by only one chemist, the figures given represent results on samples as received by the analyst.

Conclusions.

It is hardly possible to arrive at any definite conclusion from figures representing the work of so small a number of analysts. The results, however, are at least sufficient in number and agreement to encourage further investigations of the methods for determining organic nitrogen activity. Four of the five sets of results by the neutral permanganate method are in close agreement, while the fifth set is not unreasonably out of line with the others, indicating that this method is reasonably free from sources of error and gives fairly uniform results in the hands of different analysts without regard to the nature of the material upon which it is used.

Results by the alkaline permanganate method are not so uniform. The extreme difference on the sample of cottonseed meal is 1.18 per cent, the lowest percentage of availability being 44 and the highest 61. On the sample of hide and skin meal the extreme difference is 1.7 per cent, the lowest percentage of availability being 46 and the highest 66. On the samples of treated leathers and mixed fertilizers, however, this method gives uniform results in the hands of different analysts. By its use different chemists secure uniform results when working upon certain classes of materials, but upon other kinds of organic fertilizers the results are not so comparable.

In view of the importance of an early adoption of some method which will give some indication of the activity of organic nitrogen, it is recommended—

(1) That the association continue the study of the neutral and alkaline permanganate methods for organic nitrogen availability.

(2) That the suggested method for determining nitrogen in nitrates (Bul. 152, p. 28, Cir. 90, p. 2) be further studied.

A NOTE ON THE WORKING OF THE NEUTRAL PERMANGANATE METHOD.

By John M. McCandless.[1]

It has been noticed in the case of treated leathers and some other materials that after washing out the water-soluble nitrogen, if the material is treated with a little dilute ammonia water, or other alkali, a quantity of soluble matter passes into solution, coloring the solution a deep brown or black. The same phenomenon occurs with soils rich in organic matter of humus, and in peats. In the case of the two latter it is unquestionably humus which passes into solution. As is known, the official method for the determination of humus in soils calls for treatment with a 4 per cent ammonia solution, and the method for humic nitrogen calls for treatment with a 3 per cent caustic soda solution.

In Street's method, when sodium carbonate is used, this same solution of humus, or of organic matter in a physico-chemical state analogous to humus, occurs. Therefore, it is manifestly unfair to a material when sodium carbonate is added in the neutral permanganate method not to remove the soluble matter produced by the sodium carbonate, before proceeding with the digestion with permanganate. Evidently the humus-like matter brought into solution by the sodium carbonate will reduce the permanganate at once before any of the solid animal and vegetable tissue in the material can be attacked by the now weakened and partially reduced permanganate.

Since the value of humus nitrogen is generally admitted, it seems to us that the modification of Street's method used by us in the experiments on the official samples should be introduced after proper investigation.

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[1] Presented by B. B. Ross.

This modification is as follows:

After washing out the water-soluble nitrogen, transfer the residue from the filter paper with as little warm water as possible into a tall narrow beaker of about 300 cc capacity, add 1 gram of sodium carbonate, sufficient boiling water to bring to a volume of about 100 cc, stir from time to time for about two hours, dilute to about 300 cc, cover and allow to stand overnight. In the morning decant the supernatant liquor, add about 200 cc of water, and as soon as it has settled clear, decant again, then proceed as in the regular Street method by addition of permanganate solution and digestion.

Comparison of results by Street method and modified method.

Sample No.	Total nitrogen.	Insoluble nitrogen.		Availability.	
		Street method.	Modified method.	Street method.	Modified method.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	7.06	0.34	0.27	95.18	96.17
2	6.81	1.24	.14	81.78	97.94
3	8.05	1.44	.23	82.11	97.14
4	3.09	.30	.24	90.29	92.23

REPORT ON POTASH.

By H. B. McDonnell, *Referee.*

The potash work this year has been a further study of the proposed change in making the solution and of the gravimetric cobalti-nitrite method, as directed by the association, as well as a trial of the perchlorate method, as used by the Kali Syndicate, with minor changes as to quantities used. Owing to pressure of work the referee was unable to start the work until about July 1, when some preliminary work was done by Cornelius Beatty.

Fifteen chemists requested samples for the work, but, owing to the limited time before the meeting, returns have been received from but five.

Sample 1, commercial kainit, was used as the basis for the work. As received it was too wet to be properly prepared and was partially dried. Owing to the large amount of salts other than potash salts present, it was thought to be better adapted to test methods for practical use than the pure salts, although having the disadvantage of taking up moisture readily when exposed to the air.

The following letter of instructions was sent out with the samples:

Instructions for Potash Work, 1912.

Sample 1: Commercial kainit, which was partly dried to facilitate preparation. Should be weighed quickly, as it takes up moisture when exposed to the air.

Sample 2: A mixture of Sample 1 with acid phosphate, both to be weighed out by the analyst at the time of analysis. The potash in the acid phosphate is such a slight trace that it may be neglected. Calculate results on basis of kainit taken.

SAMPLE 1.

Determine potassium oxid in Sample 1 by (a) official method, (b) gravimetric cobalti-nitrite method, (c) perchlorate method. Use 2.5 grams for a 250 cc flask or 5 grams for a 500 cc flask. Use 50 cc for each determination.

(b) *Gravimetric cobalti-nitrite method, as previously used.*

Sodium nitrite solutions: Dissolve 220 grams of sodium nitrite in water and dilute to 500 cc.

Cobalt acetate solution: Dissolve 113 grams of cobalt acetate in about 300 cc of water, add 100 cc of glacial acetic acid, and dilute to 500 cc.

Sodium cobalti-nitrite solution: Mix equal parts of sodium nitrite and cobalt acetate solution a few hours before required for use. Filter just before using.

Determination: Measure with pipette 50 cc of filtered solution of Sample 1, corresponding to half a gram of kainit, into a dish, preferably of platinum, add 1 cc glacial acetic acid and an excess (10 to 15 cc) of cobalti-nitrite reagent; evaporate on a steam bath to a thick sirup, which becomes just firm on cooling. Stir with cold water until excess of cobalti-nitrite reagent is dissolved, filter, using a Gooch crucible, wash thoroughly with cold water and finally 4 or 5 times with 80 per cent alcohol. Dry to constant weight in a steam or water oven. Factor for potassium oxid, 0.2075.

(c) *Perchlorate method for kainit.*

Boil the charge with water, as in the official method, add a few drops of hydrochloric acid and barium chlorid in slight excess, cool, make to mark, and shake. Filter and transfer 50 cc of the solution to a dish, preferably of platinum, add about 1½ times as much perchloric acid as necessary to combine with all metals present, evaporate on steam bath until white fumes of perchloric acid appear; cool, add about 30 cc of 95 per cent alcohol, stir and filter through a Gooch crucible, wash with 95 per cent alcohol containing 1 cc of perchloric acid (specific gravity, 1.12 in 200 cc of alcohol), and finally 2 or 3 times with pure alcohol, using but 2 or 3 cc each time; dry precipitate in air or water oven to constant weight at temperature not over 120° C. Factor for potassium oxid, 0.34. (German Kali Syndicate method slightly modified. See also J. Amer. Chem. Soc., 1899, *21:* 33.)

The perchloric acid available is generally of specific gravity 1.12 and contains about 28 per cent acid. About 5 cc should be sufficient for this determination.

SAMPLE 2.

Weigh out 1.25 grams of kainit and 2 grams of acid phosphate if a 250 cc flask is used and twice these amounts if a 500 cc flask is used. Determine potash by (a) official method, (b) official method with modified method of making solution, (c) gravimetric cobalti-nitrite method, (d) gravimetric cobalti-nitrite method modified by Itano, (e) perchlorate method.

(b) *Official method with modified method of making solution.*

After weighing the kainit (1.25 grams) and acid phosphate (2 grams), mix them thoroughly in a small beaker, transfer to a 12½-centimeter filter with the aid of a jet of hot water, wash with successive portions of hot water into a 250 cc graduated flask to a volume of about 200 cc; add 2 cc of concentrated hydrochloric acid, heat to boiling, and add ammonium hydroxid and ammonium oxalate, and proceed as in the official method.

(c) *Gravimetric cobalti-nitrite method.*

Proceed as in the official method until after the addition of 1 cc of 1 to 1 sulphuric acid and ignition. Dissolve residue in about 30 cc of hot water and 1 cc of glacial acetic acid, add cobalt solution in excess (about 10 cc), and proceed as with kainit.

(d) *Gravimetric cobalti-nitrite method with Itano's method of making solution to remove phosphates.*

Boil sample with water as in the official method, make slightly alkaline with ammonium hydroxid, add 5 cc of milk of lime (strength not stated; suggest 5 cc for 250 cc flask or 10 cc for 500 cc flask, of a mixture with 10 grams of calcium oxid to 100 cc volume); allow to stand on hot plate (or steam bath) for one hour; add ammonium oxalate in slight excess, cool, make up to volume, filter, take 50 cc and proceed as in (c).

(e) *Perchlorate method.*

Proceed as in official method until after evaporation and ignition; dissolve residue in about 25 cc of hot water with a few drops of hydrochloric acid, add barium chlorid solution in slight excess, filter into an evaporating dish, washing precipitate, and filter with hot water; add perchloric acid in excess (about 4 cc), evaporate, add strong alcohol, filter, wash, dry, and weigh as with kainit. Note that in the perchlorate method the potash, etc., must be in the form of chlorids.

It is requested that members of the association test the proposed change in making solution (by washing out with hot water) on some of their own samples, as this modification is to come up for final adoption at the next meeting.

Please report results not later than September 1.

H. B. McDonnell, *Referee.*

Results of Collaborative Work.

Table 1.—*Comparative results on potash (percentages).*

Analyst.	Sample 1, kainit.			Sample 2, kainit and acid phosphate.				
				Platinum.				
	Official method.	Cobaltinitrite method.	Perchlorate method.	Official method.	Provisional method.	Cobaltinitrite method.	Cobaltinitrite method (Itano modification).	Perchlorate method.
H. B. McDonnell, College Park, Md.	15.37	15.54	15.24	15.18	15.47	14.92	14.92	14.57
	15.14	14.34	15.20	15.06	15.16	14.71	15.65	13.94
	15.04	.....	15.20	.....	14.98	.....	.....	14.23
	.....	.....	14.74	.....	.....	.....	.....	.....
	.....	.....	14.65	.....	.....	.....	.....	.....
Average	15.18	.....	.....	15.12	15.07	.....	.....	.....
A. G. Durgin, Orono, Me.	15.26	14.68	15.64	15.20	14.92	14.44	13.56	13.32
	15.28	14.90	15.67	15.00	15.20	14.44	14.12	13.40
	.....	14.33	.....	.....	.....	.....	.....	.....
	.....	14.33	.....	.....	.....	.....	.....	.....
Average	15.27	.....	.....	15.10	15.06	.....	.....	.....
J. B. Robb, Richmond, Va.	.....	.....	.....	14.88	15.04	.....	.....	.....
	.....	.....	.....	14.84	15.04	.....	.....	.....
	.....	.....	.....	14.80	14.96	.....	.....	.....
Average	.....	.....	.....	14.84	15.01	.....	.....	.....
Arao Itano, East Lansing, Mich.	.....	15.06	14.55	14.87	14.83	17.08	14.69	15.20
	.....	14.99	14.66	14.93	15.03	17.08	14.67	15.16
	.....	.....	.....	15.14	.....	18.64	15.07	.....
	.....	.....	.....	15.04	.....	18.40	15.15	.....
	.....	.....	.....	.....	.....	15.78	15.28	.....
	.....	.....	.....	.....	.....	15.82	15.16	.....
	.....	.....	.....	.....	.....	18.64	.....	.....
	.....	.....	.....	.....	.....	18.40	.....	.....
Average	.....	.....	.....	15.00	14.93	.....	.....	.....
H. P. Holman, Washington, D. C.	15.52	.....	.....	15.33	[1] 15.23	.....	.....	.....
	15.32	.....	.....	15.36	[1] 15.40	.....	.....	.....
	.....	.....	.....	.....	14.98	.....	.....	.....
Average	15.42	.....	.....	15.35	15.20	.....	.....	.....
B. F. Robertson, Clemson College, S. C.	15.26	.....	.....	14.98	15.11	.....	.....	.....
	15.19	.....	.....	14.79	15.21	.....	.....	.....
	15.20	.....	.....	14.79	15.03	.....	.....	.....
Average	15.22	.....	.....	14.85	15.12	.....	.....	.....
General average	15.27	.....	.....	15.04	15.06	.....	.....	.....

[1] 500 cc flask.

DISCUSSION OF RESULTS.

The results obtained by the platinum method are very satisfactory. Calculating the potash in the mixed fertilizer to the kainit basis magnifies the shortage to 0.23 per cent, as per average shown, but upon reducing this to the basis of the potash in the average mixed fertilizer it becomes of little consequence. The proposed change in making solution gives, on the average, only 0.02 per cent more, which is practically the same as the present official method, and, so far as concerns these results, indicates that the change would not be a remedy for low results. I have not been able to understand why the solution, in this method, is boiled with hydrochloric acid. It is generally clear, but may be cloudy when fresh acid phosphate is used in the mixture, owing to reversion of some of the phosphate, but as the next step is to add ammonia for this very purpose, I fail to see the reason for it.

The results by the cobalti-nitrite method are unsatisfactory and indicate that the method, at least in its present form, is not reliable. One analyst gets some good results by his modification, removing the small amount of phosphate present in the solution with milk of lime. With this method the filtrate has, as a rule, so little acid in it that it gives a precipitate with any soluble phosphate. My opinion is that the easiest remedy is to add two or three drops of strong acetic acid (enough to give a filtrate that will not give a precipitate with sodium phosphate) before filtering. I rewashed a precipitate weighing 0.1755 gram with 100 cc of such a filtrate, finishing with water and alcohol, the loss in weight being 0.0022 gram. A serious objection to this method is the unstable character of the reagent, which must be prepared "several" hours before use and is unfit for use after "several" days.

Our limited experience with the perchlorate method is also unsatisfactory, the majority of the results being low. I obtained several very high results not tabulated; they were probably due to the use of an excess of barium chlorid. Strong alcohol is a poor solvent for barium chlorid and sodium chlorid. Washing the precipitate of a blank on 1 gram of barium chlorid with the alcohol wash more than in an ordinary determination gave a residue of 0.4666 gram; rewashing this residue with 100 cc of the alcohol wash for about 10 minutes reduced the residue to 0.4190 gram. A blank on 0.5 gram of pure sodium chlorid gave a residue of 0.0169 gram.

COMMENTS OF ANALYSTS.

B. L. Hartwell, Rhode Island: We have made a number of comparisons in the past of the official method and the proposed change, but failed to find any appreciable difference between the two methods. Personally, therefore, we do not see anything to be gained by making the change. Furthermore, it will interfere with the volumetric determination of chlorin, which it has been our custom to make in potash solutions.

A. G. Durgin, Maine: I am unable to handle either the perchloric acid method or the cobalti-nitrite method in such a manner as to get results that concord with those obtained by the official method.

J. B. Robb, Virginia: I have made a comparison of the modified method of making the solution (washing out with hot water) with the official method, and, judging from a few determinations, the modified method seems to have the advantage, although the results on the mixed fertilizers seem to be about the same. The latter determinations were not made in duplicate, so the slight variance may be due to the manipulation of the methods and not to the methods themselves.

Percentage of potassium monoxid.

Method.	Sample 1.	Sample 2.	Sample 3.	Sample 4.
Official	4.76	4.22	4.60	1.98
Modified	4.70	4.30	4.70	1.88

Arao Itano, Michigan: I wish to call your attention to an omission in the direction sheet concerning Itano's modification. After precipitating the phosphate with milk of lime and after standing on the hot plate 1 hour, enough ammonium oxalate should be added to precipitate the excess of calcium. Our experience shows that the gravimetric cobalti-nitrite method gives satisfactory results when no phosphates are present, but gives high and inconsistent results when they are present. With our method of making solution to remove phosphates, however, fairly close and consistent results are obtained. The official method with modification in making solution gives very satisfactory results.

Sample 3059, official method, 11.34 and 11.23 per cent; modified method, 11.47 and 11.49 per cent.

F. P. Veitch, Washington, D. C.: It appears from these figures (see Table 1) that washing on the filter to a volume of 250 cc does not give as high results as washing to a volume of 500 cc. This confirms results which I obtained several years ago in the study of different washing solutions on fertilizers containing potash salts. My

experience with the modified method of washing on the filter has been that in many instances it gives higher potash than the old official method, but in other cases it gives practically identical results; in other words, it does not give uniformly higher results.

W. J. Jones, jr., Indiana: I regret that we shall not be able to do the cooperative work, because I should have liked to do some work on the proposed optional method for potash, to the adoption of which at this time I am very much opposed. It does not seem to me that the work which has been done with this method has given results of such a character as to justify its adoption. Furthermore, the trouble in the potash determinations is undoubtedly due to a chemical reaction which takes place in the process of manufacture, principally in what is known as 10 to 5 goods, resulting in a reversion of the potash to water-insoluble form, and the proposed method in my hands has not given theoretical results; hence, under the circumstances, it is merely a makeshift, and it seems to me that before adopting a method which cuts down the amount of material used one-fourth, greatly multiplies the working error, and adds increased manipulation, we should undertake a thorough investigation and try to discover the reason for this apparent reversion and then formulate a satisfactory method.

R. N. Brackett, South Carolina: Compared the official method of determining potash with the proposed modification with 73 samples. The results were the same by the two methods in 6 cases; higher by the official method in 25 cases; higher by the provisional method in 42 cases. In these 42 cases the difference in 8 samples was less than 0.1, in 13 samples as much as 0.1, in 7 samples 0.2, in 6 samples 0.3, in 3 samples 0.5, and in 1 sample 0.6.

Cornelius Beatty, Maryland: Analyzed a sample of fertilizer for potash by three methods: Official method, 5.29 and 5.25 per cent; gravimetric cobalti-nitrite method, 5.38 and 5.37 per cent; perchlorate method, 4.98 and 5.02 per cent. Also compared the use of denatured alcohol with ordinary alcohol in several cases, the results showing no difference.

REPORT ON AVAILABILITY OF POTASH.

By E. E. Vanatta, *Associate Referee.*[1]

On the fertilizer market in some States are found mixed fertilizers the basis of which is ashes made from burning at a low heat the coarse manure from stockyards. A large portion of the nitrogen passes off as ammonia and is combined with sulphuric acid as at the gas works. The potash and phosphoric acid are in the ashes, which react decidedly aklaline. The solubility of the potash seems to vary inversely with the degree of heat employed at the furnaces. The ash is more than 50 per cent silica and about 85 per cent insoluble in distilled water. The percentage of potash soluble in distilled water is frequently much lower than the total potash.

Several samples of fertilizers known to contain manure ashes were used for the determination of both water-soluble and insoluble potash, the former by the official method and the latter by the J. L. Smith method. The following results were obtained:

Table 1.—*Results on determination of potash in fertilizers containing manure ashes.*

Laboratory No.	Potash by official method.	Potash by J. L. Smith method.	Total potash soluble in distilled water.	Potash on long boiling in distilled water.	Increase on long boiling.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1044	2.331	4.928	47.301	2.374	1.845
1048	1.809	5.598	32.315	2.030	12.217
942	2.691	8.179	32.901	3.229	19.993
941	1.788	5.402	33.099	2.198	22.931
940	1.061	4.373	24.263	1.456	37.229
943	1.253	3.609	34.719	1.518	21.149
[2]	2.46	.....	.....	2.65	7.224
[2]	5.10	.....	.....	5.30	3.922
[2]	3.63	.....	.....	4.45	22.590

[1] In the absence of the associate referee the report was presented by Mr. Trowbridge.
[2] Determinations from J. T. Willard, of Kansas.

Study of the above data shows that in every case less than half of the total potash was soluble by the official method. It has been claimed by some that the results obtained on 30 minutes' boiling (official method) do not truly represent the water-soluble potash. For comparison the above samples were subjected to long boiling (10 to 20 hours) in distilled water. The results are shown in the fourth and fifth columns of Table 1.

In this connection it will be of interest to compare the results of some determinations made in this laboratory on the ashes from leaves and twigs of the peach and portions of the timothy plant.

TABLE 2.—*Potash determinations on ashes from peach leaves and twigs and timothy.*

Ashes from—	Laboratory No.	Potash insoluble in distilled water (J. L. Smith method).	Potash soluble in distilled water (official method).	Total potash.	Total potash soluble in distilled water.
Peach:		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Leaves	511	0.730	23.825	24.555	97.027
Do	512	.776	27.415	28.191	97.247
Do	513	.540	28.495	29.035	98.140
Do	514	.624	29.375	29.999	97.920
Do	515	.570	23.830	24.400	97.664
Do	516	.621	25.805	26.426	97.650
Twigs	517	1.688	16.515	18.203	90.727
Do	518	2.702	12.285	14.987	81.971
Do	519	1.273	9.560	10.833	88.249
Do	520	2.574	12.735	15.309	83.186
Timothy:					
Heads	728	5.115	10.638	15.753	67.530
Stalks	727	2.598	19.253	21.851	88.110
Stubble	729	2.917	20.723	23.640	87.661
Bulbs	718	1.064	34.637	35.701	97.019

All of the above samples show a high percentage of the total potash to be soluble in distilled water. They were relatively free from all adherent dirt, though the samples were not washed before ashing except in the case of the bulbs of timothy. It seems quite probable that the large amount of sand and dirt in the manure may be the cause of the low solubility of the potash. If the furnaces are run a little too hot more of the potash becomes insoluble. The referee is of the opinion that an insoluble silicate is formed, and that such potash may be no more available than that already present in the soil.

In the hope of getting some further information, three samples were prepared containing a known amount of soluble potash (7 per cent), mixed thoroughly with fine sandy soil. To samples B and C starch was added and in sample C one-third of the weight of calcium carbonate (calculated for calcium oxid) was used for part of the sandy soil. All of the samples were ignited to a white heat to burn off the starch and to drive off the carbon dioxid from sample C. These samples were then put through a sieve and thoroughly mixed as for fertilizers. Portions of each were sent to five laboratories that had offered to cooperate, with directions to analyze for water-soluble potash by the modified method.

In the Missouri laboratory we weighed out duplicates to wash with hot water, and, failing to get close agreement with the duplicates, we drew a second portion from each filtrate for the determination of the potash and obtained close agreement, showing that the trouble was with the sample itself.

We then mixed the samples very thoroughly and weighed out one from each, again mixed very carefully and weighed out a second sample from each. The results show that it is practically impossible to get a thoroughly uniform sample of such substances

when ground only to pass a millimeter mesh sieve. The reports from the collaborating laboratories show a close agreement in duplicates, but differ from each other and from our own determinations, illustrating still further the lack of uniformity of samples.

All three samples contained the same total percentage of potash, but in all cases B and C showed a decrease in the amount of water-soluble potash. This decrease is not as great as was found in the manure ashes. The results are shown in the following table:

TABLE 3.—*Analysis for water-soluble potash by the modified method.*

Analyst.	Soluble potash.			Analyst.	Soluble potash.		
	Sample A.	Sample B.	Sample C.		Sample A.	Sample B.	Sample C.
E. E. Vanatta, Missouri	5.89	3.76	5.06	H. D. Haskins, Massachusetts	6.94	6.53	5.35
	5.92	3.81	5.04		6.84	6.59	5.27
	6.61	5.28	4.87	Miss Emily Bresee, Wisconsin	7.68	7.42	4.77
	6.47	.....	.....		7.68	7.40	4.82
	5.96	4.24	5.41	E. G. Proulx, Indiana	7.62	6.04	4.98
	5.91	4.14	5.47		8.08	6.20	5.20
	5.52	4.96	5.47		7.88	6.18	5.10
	5.63	5.05	5.48				

In a letter containing the report of the Indiana station, Mr. Jones stated that it is more difficult to get a representative analytical sample by using 2.5 grams than by the official method of 10 grams and that this is particularly true of samples A and B. Our experience in this laboratory bears out this statement.

It is recommended that the referee for next year study by pot or plot experiments the relative availability of the potash from different sources, comparing especially the potash of known organic origin with inorganic forms.

REPORT ON SOILS.

By G. S. FRAPS, *Referee.*

The following instructions were sent out to those who had signified their intention of cooperating in the work on soils in 1912:

INSTRUCTIONS FOR WORK ON SOILS, 1912.

The work on soils embraces three topics of study: (1) Acidity, (2) method of extracting the humus, and (3) comparison of the Rather method with the official method for humus.

Four samples are sent out, Nos. 5961 and 5962 for the acidity work and Nos. 5974 and 5975 for humus work. Nos. 5961 and 5962 are soils furnished by B. L. Hartwell from the Rhode Island experiment station.

DETERMINATION OF ACIDITY.

Lime water.—Place 10 grams of quicklime in 2,000 cc of water and allow to stand at least 24 hours, shaking every hour; filter, and protect from the air in glass-stoppered bottles. Titrate 50 cc with fifth-normal acid and 1 cc of phenolphthalein until color just disappears; calculate the quantity of calcium oxid per cubic centimeter. One cubic centimeter of fifth-normal acid is equivalent to 0.0056 gram of calcium oxid. The strength of the lime water must be determined from time to time, as it is likely to take up carbon dioxid from the air and become weaker.

Determination.—Weigh out 5 grams of soil and place in a small evaporating dish; add the required amount of standard lime water, then 50 cc of water, and evaporate the solution to dryness on the water bath. Wash it into an Erlenmeyer flask with 100 cc of water and allow to stand 24 hours. Pipette off 50 cc, taking care not to disturb the soil. Filter if necessary in order to get a clear solution. Add 1 cc of phenolphthalein

and boil the solution until a pink color appears, or to 5 cc if no color appears. The results are calculated as parts per million of calcium oxid required to neutralize the soil. Jena glassware must be used, as ordinary glassware may give an alkaline reaction when boiled with water.

In estimating the acidity of a number of soils, it is best to test first for acidity or alkalinity on all the samples at once. Treat such soils as are acid with 1 cc of lime water as directed above, those acid to 1 cc with 2 cc, and so on with 5, 10, 20, and 50 cc. Then make other tests to narrow the limits of the method. This method is much quicker than running a number of tests with different amounts of lime water at the same time upon the same soil.

Please report (a) quantity of lime water at which the soil is alkaline and acid, and (b) limits within which the soil is alkaline or acid, in parts per million of calcium oxid.

METHOD OF EXTRACTING THE HUMUS.

Please compare the results by the three following methods on Soils 5974 and 5975.

Official method.—Place 10 grams of the sample in a Gooch crucible, extract with 1 per cent hydrochloric acid until the filtrate gives no precipitate with ammonium hydroxid and ammonium oxalate, and remove the acid by washing the soil with water. Wash the contents of the crucible (including the asbestos filter) into a glass-stoppered cylinder with 500 cc of 4 per cent ammonium hydroxid and allow to remain, with occasional shaking, for 24 hours. During this time incline the cylinder as much as possible without bringing the contents in contact with the stopper, thus allowing the soil to settle on the side of the cylinder, and exposing a very large surface to the action of the ammonium hydroxid. Place the cylinder in a vertical position and leave for 12 hours to allow the sediment to settle, filter the supernatant liquid (the filtrate must be perfectly clear), determine the organic matter and ash by the Rather method.

Snyder's method.—Weigh 10 grams of the soil into a wide-mouthed cylindrical bottle of about 500 cc capacity, provided with a ground-glass stopper. Add to the soil 100 cc of the 1 per cent hydrochloric acid cautiously, lest the carbonates cause frothing over; stopper and shake from time to time during the day. Allow to stand overnight, decant o he liquid through a filter when necessary, and add 100 cc of the hydrochloric acid to the residue, using a part of the acid to rinse back from the paper to the bottle any adhering soil. Shake the bottle from time to time and again allow to stand overnight and again decant. Repeat until all the lime is extracted from the soil. Wash the soil from the bottle onto a filter paper, and when washed thoroughly free of hydrochloric acid wash the soil back into the bottle, using 100 cc of 4 per cent ammonia. Shake every half hour during the day and allow to stand overnight. In the morning decant the liquid off into bottles of 1-liter capacity. Add to the soil in the bottles another 100 cc of ammonia and again shake from time to time during the day and allow to stand overnight and decant off in the morning. Repeat the extracting with ammonia until all the humus is removed and the extract has little color. Pour the extracts into a 500 cc graduated flask, make up to the mark with distilled water, and allow to settle for one day. Pour off about 400 cc of the solution without disturbing the sediment in the bottom. Shake the solution thoroughly before removing each aliquot for analysis. Determine the organic matter and ash by the Rather method.

Modified official method.—Digest 10 grams of soil for five hours with 200 cc of water and 20 cc of concentrated hydrochloric acid (1.20 specific gravity). Filter on a fluted filter and wash thoroughly; cover and allow to drain overnight. Measure out 500 cc of 4 per cent ammonia (1 cc=11.55 cc fifth-normal hydrochloric acid) and wash the soil into a glass-stoppered bottle with the ammonia. Digest for 24 hours, shaking every hour during the working day; let settle 24 hours, decant about 400 cc through a folded filter into a dry glass-stoppered bottle, and use aliquots of the solution for the analysis. Shake the bottles well before withdrawing samples for the analysis. Determine humus and ash by the Rather method.

COMPARISON OF THE RATHER AND OFFICIAL METHODS.

With limited time prepare the solution by the modified official method and determine organic matter and ash by the methods given below. If solutions are prepared by all three methods given above, please compare the two methods on all these solutions.

Official method.—Evaporate 100 cc of solution to dryness in a platinum dish, dry for four hours in a boiling-water oven, and weigh. Ignite, and weigh again. Report loss on ignition, and ash.

Rather method.—Place 130 cc in a glass-stoppered cylinder and add 0.65 gram of ammonium carbonate. Allow to stand overnight, filter through a dry filter into a dry flask, and measure out 100 cc for evaporation in the platinum dish. Complete as in the official method.

Please report percentages of loss on ignition and ash on the air-dry sample and give us the benefit of your observations or suggestions concerning these methods.

Results of Humus Work.

The following table shows the results secured by the various analysts:

Percentage of humus in the soils by three methods.

Analyst.	Method of extraction.	Humus.				Ash.			
		Sample 5974.		Sample 5975.		Sample 5974.		Sample 5975.	
		Completed by—		Completed by—		Completed by—		Completed by—	
		Official.	Rather.	Official.	Rather.	Official.	Rather.	Official.	Rather.
J. B. Rather, Texas	Official	3.44	2.02	2.51	1.05	11.03	0.28	11.08	0.60
	Snyder	4.20	2.18	4.12	1.15	16.38	.39	30.23	.79
	Modified official	3.34	1.78	2.22	.97	13.76	.21	15.33	.60
	Official	3.63	2.18	2.20	1.19	12.55	.37	10.23	1.34
	Snyder	4.58	2.39	3.49	1.36	20.80	.58	25.90	2.15
	Modified official	3.95	1.93	2.75	.97	15.92	.48	16.47	.84
G. W. Walker, Minnesota.	Official	2.78	2.03	2.89	1.04	6.08	.34	19.23	1.05
	Snyder								
	Modified official				1.07				1.50
S. D. Averitt, Kentucky.	Official	3.19	2.14	2.75	1.02	13.32	.36	21.68	.87
	Snyder								
	Modified official	3.29	2.12	1.90	1.17	11.84	.40	12.60	.89
J. P. Aumer, Illinois.	Official	2.31	2.33	1.23	1.50	1.38	.67	2.54	1.43
	Snyder	4.82	2.44	3.77	1.18	17.69	.43	24.07	1.22
	Modified official	2.46	2.16	1.24	1.03	2.74	.50	2.62	1.91

COMMENTS BY ANALYSTS.

J. B. Rather, Texas: The official method is more convenient than the Snyder method. The removal of the lime by the former and the subsequent washing with water required about three or four weeks. The Snyder method is also very tedious. The modified method is much easier and quicker than the others, but the results are lower. It is possible that the reaction between the acid and the bases in the soil is a reversible one, and this might account for the low results in this method. I believe that freshly prepared ammonia should be used in humus determinations, as I have found old solutions to contain considerable nonvolatile solids. The fact that humus "ash" may be neither inorganic matter combined with the humus nor suspended clay, but ammonia-soluble soil minerals, should be considered in humus determinations.

G. W. Walker, Minnesota: Solution prepared as described under the official method, except that a filter, S. & S., No. 589, 11 cm, was used instead of a Gooch and no suction used, the filter being punctured and soil washed into a cylinder. In case of sample No. 5975 it was found practically impossible to use a Gooch. In preparing solution according to the modified method, it was found impossible to wash soil free from fluted filter in case of No. 5974. In my opinion the Rather method marks a distinct advance over the official method and should be adopted as the official method.

S. D. Averitt, Kentucky: The Rather and official methods are compared on the two methods of extraction (official and modified). I am very favorably impressed with the Rather method; extraction by the modified method has several advantages over the official method.

In addition to the association samples, two Kentucky soils, No. 14412, a virgin soil, rich in organic matter, and No. 25335, an average blue-grass soil, were run for humus by the same methods, with the following results:

Method and sample No.	Rather method.		Official method.		Method and sample No.	Rather method.		Official method.	
	Loss.	Ash.	Loss.	Ash.		Loss.	Ash.	Loss.	Ash.
Official method:	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	Modified method:	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
14412	4.87	1.18	5.69	9.01	14412	4.97	1.64	5.82	11.08
	4.63	1.05	5.62	10.03		4.99	1.40	5.77	11.18
25335	2.41	1.00	3.25	7.46	25335	2.67	.73	3.55	7.81
	2.41	1.05	3.16	7.65		2.60	.56	3.33	7.86

The precipitation with ammonium carbonate, as proposed by Rather, is a decided improvement, and should be adopted by the association. The method of dissolving out the humus should be subjected to further study. The present official method is very tedious. The Snyder method offers little or no advantage over the association method. The modified method is the most promising of the three, though at times it gives somewhat lower results.

RESULTS OF THE WORK ON ACIDITY.

The following are the results on the two samples:

Analyst.	Acidity in parts per million (as calcium oxid).	
	Sample No. 5961.	Sample No. 5962.
P. R. McMiller, St. Paul, Minn	3,950	3,120
Kelly & Ludlum, College Station, Tex	5,060	4,400
J. P. Aumer, Urbana, Ill	5,571	.....

COMMENTS BY ANALYSTS.

P. R. McMiller, Minnesota: In my opinion this method appears to give fairly accurate results, being far more reliable than the Hopkins method, but from a chemist's standpoint the time element must be considered when there are many analyses to be made. The number of determinations which must be made before alkalinity is reached is too great for a practical laboratory method.

The agreement between the results of the laboratories leaves much to be desired.

RECOMMENDATIONS.

It is recommended—

(1) That the Rather modification of the method for humus be adopted as official.

(2) That the methods for extracting the humus and the methods for acidity be studied further.

A paper on "A Proposed Modification of the Official Method of Determining Humus," by O. C. Smith, of the University of Missouri, was read by P. F. Trowbridge and has been published in the Journal of Industrial and Engineering Chemistry, 1913, volume 5, page 35.

A paper on the "Application of the Ammonium Carbonate Method for the Determination of Humus to Hawaiian Soils" was presented

by J. B. Rather and has since been published in the Journal of Industrial and Engineering Chemistry, 1913, volume 5, page 222.

A motion by W. A. Withers that the association appoint an associate referee on soils, to consider methods for determining ammonia, nitrates, and nitrites in soils, was carried and referred to the executive committee for consideration.

REPORT ON INORGANIC PLANT CONSTITUENTS.

By W. H. McIntire, *Referee.*

Among the recommendations of the association at its 1911 meeting to the referee on inorganic plant constituents was the study of the Herman Schreiber method for total sulphur in organic matter (Cir. 56, Bureau of Chemistry), with a view to official adoption. This method was studied by Associate Referee B. E. Curry, who reports that very good results were secured in his laboratory by three of the four analysts to whom samples of feedstuffs were assigned. Doubtless because of the early meeting of the association none of the collaborators reported to Mr. Curry.

For the study of the oxalate method for iron and aluminum and the molybdate method extended to the determination of calcium and magnesium, synthetic solutions were prepared from analyzed chemicals and aliquots sent to 10 collaborators.

In regard to the former method for iron and aluminum the referee would state that the results obtained thereby are not satisfactory. R. F. Hare stated as follows: "Our results were all low compared with the molybdate method, and the four determinations did not check each other. I am sure we did not get a complete precipitation of iron and aluminum. We could not get a precipitate with ammonium acetate as long as the solution was the least bit acid. I should not be willing to submit the results obtained by the oxalate method."

The results secured by J. H. Pettit and Mr. Weaver were likewise low, as were those obtained by Mr. Hardy and the referee. Mr. Pettit stated that "the precipitation of iron and aluminum was not complete, and on standing after filtration iron and aluminum separated out, giving the solution a cloudy appearance." As accounting in part at least for the low results on iron and aluminum, the referee found much iron as well as manganese in the ignited calcium oxalate precipitate, due to the well-known tendency of occlusion in the filter of nearly neutral solutions of these elements where the washings can not be acidified. For this reason, reinforced by two years of unsatisfactory reports and unfavorable comments, as well as by the adoption of the molybdate method, the referee recommends that cooperative study of the method be discontinued.

The extended molybdate method for iron and aluminum having worked well with small amounts of calcium and magnesium in the laboratory of the referee, the method was tried as to its applicability to large amounts by introduction of much of these elements into the official synthetic solution. The scheme as tried included the evaporation to small volume (75 cc) of the ammoniacal filtrate from iron and aluminum, to permit the elimination of manganese by bromin water, as is officially done for soils. It was found and noted by Mr. Pettit, Mr. Shedd, Mr. Hardy, and the referee that concentration to 75 cc caused precipitation of calcium or calcium molybdate. The referee and Mr. Hardy, of the same laboratory, also noted that precipitation of the calcium as oxalate in the presence of manganese from volume of 400 cc, followed by resolution and reprecipitation from the same dilution, produced excellent calcium oxid results. The calcium filtrate could then be evaporated to dryness and heated from above to expel ammonium salts and the magnesium and manganese be taken up with nitric acid, manganese precipitated therefrom as manganese dioxid by potassium chlorate, as is done in the Ford Williams method for manganese in case of

large amounts of this element. Since manganese occurrences are almost always minute and often lacking, the referee would recommend that the extended method be further studied in view of its following well the approved molybdate scheme and because of its satisfactory results when occurrences of manganese are negligible.

RECOMMENDATIONS.

It is recommended—

(1) That the referee for next year be instructed to study further the Schreiber method (Cir. 56) for total sulphur.

(2) That cooperative study of the oxalate method for iron and aluminum be discontinued.

(3) That the molybdate method extended to the determination of calcium as oxalate and magnesium as ammonium magnesium phosphate (Bul. 152, p. 62; Cir. 90, p. 5) be further studied.

(4) That the molybdate method for the separation of iron and aluminum in an ash solution (Bul. 152, p. 61; Cir. 90, p. 4) be adopted as official.

At 12.15 the convention adjourned until 1.30 p. m.

MONDAY—AFTERNOON SESSION.

At the opening of the afternoon session the following committees were announced:

Committee to invite the Secretary or the Assistant Secretary of Agriculture and H. W. Wiley to address the association: E. F. Ladd, of North Dakota; R. J. Davidson, of Virginia; and A. J. Patten, of Michigan.

Committee on resolutions concerning the services of H. W. Wiley to the association: R. J. Davidson, of Virginia; B. B. Ross, of Alabama; A. J. Patten, of Michigan; J. P. Street, of Connecticut; and E. F. Ladd, of North Dakota.

Committee on nominations: W. A. Withers, of North Carolina; J. M. Bartlett, of Maine; and William Frear, of Pennsylvania.

Auditing committee: B. L. Hartwell, of Rhode Island; W. H. McIntire, of Tennessee; and R. C. Thompson, of Arkansas.

REPORT ON INSECTICIDES.

By S. D. AVERITT, *Referee.*

The work on insecticides is a continuation of the work of last year in that it is an effort to find accurate methods for the determination of the different forms of sulphur in lime-sulphur solution, and the time, conditions, and temperature of digestion for soluble arsenic in lead arsenate.

In regard to official methods of analysis, three conditions are essential: First, the method must be accurate; second, there must be no unnecessary difficulties in the operations concerned; and, third, the time consumed must be as short as is consistent with accuracy.

The methods presented to the association last year for sulphid and thiosulphate sulphur (Bureau of Chemistry Cir. 90, pp. 6–7) gave concordant results, and it was recommended by the previous referee that they be made official. The recommendations were approved and referred to this meeting for final action.

The referee feels that these methods do not conform to the essential conditions, and that the association should not take final action on them before considering the methods which the referee proposes in this report. He believes that while the former give concordant results, they are inaccurate, long, and tedious, and that the methods now proposed are not only accurate, but are easy to work and rapid.

When made by the formulas now proposed for its manufacture, lime-sulphur solution is relatively simple, consisting of a large amount of calcium polysulphid (CaS_x), a smaller amount of calcium thiosulphate (CaS_2O_3), and very small amounts of calcium sulphate ($CaSO_4$) and calcium sulphite ($CaSO_3$); the last is present only in traces and is negligible in the analysis. When the sulphur is present in the excess now recommended the sulphid sulphur is all pentasulphid (CaS_5) or a mixture of the pentasulphid and tetrasulphid (CaS_4). From this consideration it follows that the essential determinations in the analysis are total sulphur in solution, thiosulphate sulphur, and sulphate sulphur, as the sulphid sulphur is equivalent to the difference between the total sulphur and the sum of the thiosulphate and sulphate sulphur. In order to understand the solution more thoroughly, however, it is necessary to know the total lime in solution, as the proportion of lime in the sulphid sulphur determines the exact character of the solution.

The determination of the monosulphur equivalent called for in this year's work is important in that the ratio of the total sulphid sulphur to the monosulphur equivalent affords the means of calculating the exact amount of pentasulphid and tetrasulphid sulphur present in the solution when made by formulas calling for an excess of sulphur. It is well to state in this connection that the tendency is to make the higher sulphids with the pentasulphid as the limit.

The methods proposed for lime-sulphur solution this year are those used by J. E. Harris of the Michigan station (published in Technical Bulletin No. 6 of that station), with certain changes in manipulation and detail which were found to facilitate the work. An extract from a letter written to the former referee while I was associate and the letter written to the cooperators in this year's work will explain the reason for the present work on lime-sulphur solution.

The results for sulphid and thiosulphate sulphur show good concordance, but I believe they are incorrect figures for the solutions submitted.

The Geneva (N. Y.) station and other investigators have shown that an atom of calcium can hold but 5 atoms of sulphur (CaS_5), but the ratio of the total sulphid sulphur to the sulphur in calcium monosulphid is 5.4 in solution 1, and 5.6 in solution 2. I am unable to understand how there can be as much sulphid sulphur in these solutions as the method shows.

The thiosulphate figures are, I think, incorrect, and these two points should have more study. I am submitting some figures which I think will prove interesting to you in view of two well-established facts: First, that no sulphid higher than CaS_5 can be formed; second, that there is no calcium hydrate in the solution. In a straight lime-sulphur solution the lime as calculated from the iodin titrations plus that in the calcium sulphate should check the determined lime.

Instructions for Cooperative Work of 1912 on Insecticides.

Instructions for the work were sent out March 20, 1912. During the next few days after sending out samples and instructions, some further work was done on sulphid sulphur and some changes made in the methods as at first proposed. These changes were communicated to the cooperators in a letter dated March 27, 1912, which is included as a part of the instructions.

March 20, 1912.

I am sending you two samples of lime-sulphur solutions and one of arsenate of lead for A. O. A. C. work on insecticides, and inclosing instructions for that work.

After considerable work done last year and during the last few days I am thoroughly convinced that the methods proposed as official for sulphid and thiosulphate sulphur in lime-sulphur solutions (see Bureau of Chemistry Cir. 90, pp. 6-7) give incorrect

results, and as referee I desire to get before the association methods that are direct and accurate.

Before beginning the work last year I had read Technical Bulletin No. 6 of the Michigan station, issued in January, 1911. The methods employed by Mr. Harris in that work seemed direct and practical, and on the 1911 A. O. A. C. samples of lime-sulphur and on others made by myself I verified to my entire satisfaction the methods given in that bulletin, and I want them tested by others, with a view of presenting them to the association for consideration as official methods.

If you can not find the above bulletin in your station library advise me at once and I will see that you are provided with a copy, as I want you to read it before doing the work on the lime-sulphur solutions. Keep all samples protected from the air as much as possible.

Report results as soon as possible after the work is done, as the referee has to send an abstract of his report to the committee three weeks before the date of the meeting.

Any assistance you may render in this work will be appreciated.

S. D. Averitt, *Referee on Insecticides.*

Two samples of lime-sulphur solution and one sample of lead arsenate were sent out for cooperative work. The lime-sulphur solutions were made as follows:

No. 1.—40 grams of good lime and 90 grams of sulphur were ground together and boiled 50 minutes with 450 cc of distilled water with a reflux condenser, cooled somewhat, filtered and diluted to 600 cc with distilled water, let stand about 10 days in a glass-stoppered bottle, then filtered into small bottles and sealed.

No. 2.—No. 2 was made and treated in exactly the same way except that 30 grams of lime and only 37½ grams of sulphur were used. It was intended that the sulphid sulphur in No. 1 should all be pentasulphid (CaS_5) and that in No. 2 it should be a mixture of pentasulphid and tetrasulphid. Subsequent analyses show that in No. 1 all the sulphid sulphur is pentasulphid and that in No. 2 about 40 per cent is pentasulphid and the remainder is tetrasulphid.

The sample of lead arsenate sent out was commercial lead arsenate paste.

LIME-SULPHUR SOLUTIONS.

Total sulphur in solution.—Weigh accurately about 5 grams of No. 1 and 10 grams of No. 2. Make to 100 cc and use 10 cc aliquots for each determination. (In the case of concentrates 5 grams should be made to 200 cc and 10 cc aliquots used.) Transfer the aliquots to a wide-neck Erlenmeyer flask. Add 3 grams sodium peroxid and cover immediately with a watch glass and heat on steam bath for 5 or 6 minutes after shaking it thoroughly. Dilute somewhat and add 10 cc concentrated hydrochloric acid. Make to about 125 cc. Boil a few minutes and add slowly with thorough shaking 10 to 15 cc of a 10 per cent solution of barium chlorid. Calculate the sulphur from the weight of barium sulphate. If it is desired to filter the barium sulphate on standing an hour or two, 2 or 3 cc more hydrochloric acid should be added.

Note.—A good grade of sodium peroxid is usually free from sulphur, but should be tested and a blank run if sulphur is found. The method is changed from that recommended last year, in order that the conditions under which the precipitation of barium sulphate is made may be more uniform.

Monosulphur equivalent ("monosulphid sulphur" Technical bulletin No. 6, Michigan station. It is unfortunate that the term "monosulphid sulphur" should have been used in this connection, as there is no monosulphid sulphur in the solution).—Dilute 10 cc of solution prepared for total sulphur to about 60 cc in a wide-neck Erlenmeyer flask and titrate with tenth-normal iodin until the yellow color of the polysulphids disappears. The precipitated sulphur acting as a background gives a white solution. From the number of cubic centimeters of tenth-normal iodin calculate the monosulphur equivalent $I_2=S$ (1 cc N/10 I=0.0016 S).

Thiosulphate sulphur.—Continue the above titration carefully, letting the iodin act as its own indicator until a small drop produces a very slight permanent coloration. The additional iodin represents thiosulphate sulphur, according to the following reaction:

$$2\ CaS_2O_3+I_2=CaI_2+CaS_4O_6\ (1\text{ cc N/10 I}=0.0064\text{ S}).$$

Sulphate and sulphite sulphur.—To the filtrate (without the washings) from the thiosulphate determination add several drops of hydrochloric acid and precipitate in the cold with about 10 cc of 10 per cent barium chlorid, shaking thoroughly, and let

stand overnight in the cold. From the weight of barium sulphate calculate the sulphate and sulphite (now sulphate) sulphur.

NOTE.—The precipitated sulphur should stand several hours before filtering. Filter on a 7 cm ashless filter with moderate suction.

Total sulphid sulphur.—Direct determination: Place the filtered and washed sulphur from the thiosulphate determination with the filter paper in a beaker or flask and dissolve the sulphur in 10 cc (1 to 2) sodium hydroxid solution (boiling to dissolve the sulphur), then add 25 to 30 cc of 3 per cent hydrogen peroxid, and heat on steam bath one-half hour. Make slightly acid with hydrochloric acid and filter to get rid of paper; wash to about 125 cc with hot water, add 3 cc of concentrated hydrochloric acid, boil and precipitate with 10 to 15 cc of 10 per cent barium chlorid. Let stand overnight. From weight of barium sulphate calculate sulphid sulphur. A blank must be run on sodium hydroxid and hydrogen dioxid and correction made therefor.

NOTE.—Total sulphur−(thiosulphate+sulphate sulphur)=sulphid sulphur.

Total lime (CaO) in solution.—Determination: Oxidize a 10 cc aliquot with sodium peroxid as for total sulphur, make acid in the same manner, make to about 50 cc, boil a few minutes, and make faintly alkaline with ammonium hydroxid. Add dilute hydrochloric acid until it clears up, then a drop or two in excess. Now add 0.5 gram of finely ground ammonium oxalate; bring to boiling, shake a little, set aside to cool, filter, and weigh calcium oxid.

Calculation of lime from iodin titration: Monosulphur equivalent $\times 1.75$+thiosulphate sulphur$\times 0.875$+sulphate sulphur$\times 1.75$=total calcium oxid.

LEAD ARSENATE.

Water-soluble arsenic.—(1) Weigh to 0.01 gram about 2 grams of the paste; place in a tightly stoppered bottle with 200 cc carbon-dioxid-free water per gram; put in a mechanical shaker and shake for 3 hours; let stand 2 hours and filter; use 200 cc of the clear filtrate for the determinations; add 0.5 cc sulphuric acid and proceed as directed under water-soluble arsenic oxid, page 240, Bulletin 107, Revised, Bureau of Chemistry. It is important that the filtrate shall be perfectly clear and that the titration with thiosulphate and iodin be very carefully done. Make correction for amount of iodin required to produce color titrated to, using same chemicals and volumes.

(2) Same as (1), except that the bottles should be placed at some convenient place in the laboratory and shaken every half hour, or as often as possible during a working-day. Let stand overnight and filter. State temperature at which digestions are made in each case.

ADDITIONAL INSTRUCTIONS.

LEXINGTON, KY., *March 27, 1912.*

DEAR SIR: Since sending you the samples and instructions for A. O. A. C. work on insecticides, I have done some further work on total sulphid sulphur and wish to make a few changes in the method as given. Instead of 10 cc (1 to 2) sodium hydroxid solution, use 15 cc and heat on steam or water bath until the sulphur dissolves. Do not boil, as it sometimes melts the sulphur, rendering complete solution very difficult. It requires from one to one and a half hours on steam bath to dissolve the sulphur. Keep the flask covered and shake gently a few times during the digestion to get the sulphur off the sides. Use 40 cc of hydrogen dioxid or 3 grams of sodium dioxid to oxidize. In case sodium dioxid is used the oxidation will be completed in from 5 to 10 minutes.

The sulphid sulphur may be weighed directly with fairly accurate results, and as it requires so little additional time, I should be glad if you would try it.

I found that an accurate analysis of a lime-sulphur solution could be made in less than two hours, provided the sulphate sulphur, which is always small, is assumed.

The following method of weighing the sulphur from the thiosulphate determination was found to be rapid and accurate: Wash a 7 cm ashless filter two or three times, using suction; dry in water oven; cool in desiccator and weigh; filter the sulphur on this weighed filter, drying as before, weighing as quickly as possible without putting in desiccator.

The sulphid sulphur plus thiosulphate sulphur agrees very closely with the total sulphur as determined. Now assume 0.10 per cent for sulphate sulphur, which is taken from the sulphid sulphur as weighed, and you have an analysis which for practical purposes is sufficiently accurate, and the time saved outweighs the small loss of accuracy.

S. D. AVERITT, *Referee on Insecticides.*

Lime-Sulphur Solutions.

The method recommended by the previous referee for total sulphur in solution is not sufficiently definite and would lead to the precipitation of large and small amounts of barium sulphate under the same conditions as to volume, acidity, etc., and in the hands of inexperienced analysts it is likely to give varying results. For example, the difference between the highest and lowest results on sample No. 1 last year is nearly 0.4 per cent, and one analyst shows a difference in his duplicates of 0.3 per cent. The results this year show much better concordance, but there is still room for improvement. The Dow Chemical Co., of Midland, Mich., has objected to the method as adopted by the association on account of the varying results obtained by different chemists. After giving the matter considerable study, and reading Otto Folin's article on the precipitation of barium sulphate in the Journal of Biological Chemistry, 1905, volume 1, page 131, I came to the conclusion that the method as adopted by the association was deficient in detail for the best results in the hands of all workers. I therefore made the present recommendations for the determination of total sulphur. The proposed method requires no more time than the other and will give accurate as well as concordant results if followed. Sodium peroxid as used in the present method is not only a better oxidizing agent than hydrogen peroxid but saves time, and a good grade of sodium peroxid is usually free from sulphur.

Relative to the work done last year on sulphid and thiosulphate sulphur by the methods proposed at that time and the work by those now proposed, the referee offers the following brief discussion of facts and figures as they were presented to the referee last year.

The cooperative work was done on one straight lime-sulphur solution (No. 1). The other (No. 2) contained sodium thiosulphate. For that reason I shall discuss the figures for No. 1 of the cooperative samples and for another sample made by myself which I shall call No. 3.

Results on sample No. 1.

Method and analyst.	Total sulphur.	Sulphid sulphur.	Thiosulphate sulphur.	Sulphate sulphur.	Monosulphur equivalent.	Calcium oxid.
Methods proposed in 1911:	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
C. C. McDonnell	12.15	10.36	1.84	0.03	.....	.....
S. D. Averitt	12.26	10.26	1.83	.03	.....	.....
Average of all collaborators	12.40	10.40	1.83	.06	.....	.....
Methods proposed in 1912:						
S. D. Averitt	.....	9.42	2.76	.22	1.93	6.05

1.93 per cent monosulphur equivalent=3.38 per cent calcium oxid, 2.76 per cent thiosulphate sulphur=2.41 per cent calcium oxid, 0.22 per cent sulphate sulphur =0.39 per cent calcium oxid, sum=6.18 per cent calcium oxid, determined calcium oxid=6.05 per cent, difference=0.13 per cent (and the sulphate sulphur is known to be high). Using the thiosulphate and sulphate sulphur as determined by methods proposed in 1911, 1.93 per cent monosulphur equivalent=3.38 per cent calcium oxid, 1.84 thiosulphate sulphur=1.61 per cent calcium oxid, and 0.03 per cent sulphate sulphur=0.05 per cent calcium oxid, sum=5.04 per cent calcium oxid, determined calcium oxid=6.05 per cent, difference=1.01 per cent. In a straight lime-sulphur solution the lime as determined and as calculated from the iodin titrations and sulphate sulphur must check, since all the lime is in combination with sulphur as sulphids, thiosulphate, and sulphate (the calcium and not the sulphur is reacting with iodin). Is not the inconsistency of the methods proposed in 1911 patent? Further, suppose that there is 10.40 per cent sulphid sulphur as shown by these methods. The ratio of this to the monosulphur equivalent=10.40÷1.93=5.39, which would

necessitate hexasulphid sulphur (CaS_6), and such a thing does not exist. On the other hand, suppose that there is 9.42 per cent sulphid sulphur as shown by the methods proposed. The ratio of this to the monosulphur equivalent=9.42÷193 =4.9 (nearly), which is well within the limit, which is 5.

In sample No. 3, made by myself, the following results were obtained:

Results on sample No. 3.

Method and analyst.	Total sulphur.	Sulphid sulphur.	Thiosulphate sulphur.	Sulphate sulphur.	Monosulphur. equivalent.	Calcium oxid.
Methods proposed in 1911, average	*Per cent.*	*Per cent.*	*Per cent.* 1.19	*Per cent.* 0.02	*Per cent.*	*Per cent.*
Methods proposed in 1912, average	6.60	4.62	1.88	.10	0.92	3.30

0.92 per cent monosulphur equivalent=1.61 per cent calcium oxid, 1.88 per cent thiosulphate sulphur=1.64 per cent calcium oxid, 0.10 per cent sulphate sulphur= 0.17 per cent calcium oxid, sum=3.42 per cent calcium oxid, determined calcium oxid=3.30 per cent, difference=0.12 per cent. Using the thiosulphate sulphur and sulphate sulphur as determined by the 1911 methods, 0.92 per cent monosulphur equivalent=1.61 per cent calcium oxid, 1.19 per cent thiosulphate sulphur= 1.05 per cent calcium oxid, and 0.02 per cent sulphate sulphur=0.03 per cent calcium oxid, sum=2.69 per cent calcium oxid, determined calcium oxid=3.30 per cent, difference=0.61 per cent. Taking the sum of the thiosulphate sulphur and the sulphate sulphur, as determined by the 1911 methods, from the total sulphur, 6.60— (1.19+0.02)=5.39 per cent sulphid sulphur, 5.39÷0.92=5.9. On the other hand, 4.62÷0.92=5.02. From the above the appropriateness of the present recommendation relative to the analysis of lime-sulphur solution is readily seen. Several other samples of lime-sulphur solutions, including concentrates, worked the past year by me have shown excellent results by the methods proposed this year.

RESULTS OF 1912 WORK.

On account of the early date of the meeting, several of the cooperating chemists did very little work. Only two chemists besides the referee report any work on the determination of sulphid sulphur weighed as barium sulphate. Most of them weighed the sulphid sulphur directly, but the results in the main are too high.

The direct determination of sulphid sulphur is not important if the total sulphur is determined, as it may be determined by difference as noted under sulphid sulphur determinations. If it is desired, however, to shorten the analysis by determining the sulphid sulphur and taking the sum of sulphid, sulphate, and thiosulphate sulphur for the total sulphur, the method for sulphid sulphur as recommended is accurate.

As stated in the additional instructions for sulphid sulphur sent to the cooperators, an accurate analysis of a lime-sulphur solution can be made in less than two hours, and while it is not recommended this year the association should instruct the next referee to investigate the rapid method further, and if found sufficiently accurate it should be made a provisional method in consideration of the time saved.

Cooperative results on lime-sulphur solutions, 1912.

SAMPLE No. 1.

Analyst.	Total sulphur.	Monosulphur equivalent.	Thiosulphate sulphur.	Sulphid weighed as barium sulphate.	Sulphur weighed as sulphur.	Sulphate and sulphite sulphur.	Calcium oxid.	
							Determined.	Calculated.
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Geo. P. Gray, California	10.39	1.44	[1] 2.91	7.76	8.18	0.12	[1] 5.24	[1] 5.28
S. D. Averitt, Kentucky	10.32	1.51	2.56	7.60	7.69	.10	5.06	.....
	10.41	1.56	2.50	7.40	7.68	.09	5.09	.....
	10.36	1.51	2.56	7.60	7.60	.10	5.04	.....
	10.37	1.53	2.55	7.46	7.64	.09	5.06	.....
	.....	1.55	2.50	.....	7.67	.....	.....	.....
	.....	1.53	2.55	.....	7.63	.....	.....	.....
	.....	1.52	2.56	.....	7.63	.....	.....	.....
	.....	1.50	2.50	.....	.....	.....	.....	.....
	.....	1.52	2.56	.....	.....	.....	.....	.....
Average	10.36	1.53	2.54	7.52	7.65	.10	5.06	5.07
Wm. C. Marti, Michigan	10.48	1.63	2.25	8.20	8.23	.16	5.02	.....
	10.34	1.63	2.25	8.17	8.34	.10	5.09	.....
	.....	1.63	2.19	.....	.....	.....	.....	.....
	.....	1.63	2.19	.....	.....	.....	.....	.....
Average	10.41	1.63	[1] 2.22	[1] 8.19	8.29	.13	5.06	5.01
W. B. Pope, Washington, D. C	10.38	1.56	2.45	.....	8.36	Trace.	4.62	.....
	10.38	1.54	2.45	.....	8.29	.....	4.54	.....
	10.44	1.53	2.45	.....	8.19	.....	.....	.....
Average	10.40	1.54	2.45	.....	8.28	.....	[1] 4.58	4.84
C. C. McDonnell, Washington, D. C	10.30	1.47	2.92	.....	7.84	.....	4.70	.....
	10.30	1.57	2.45	.....	7.88	.....	.....	.....
	.....	1.49	2.83	.....	7.95	.....	.....	.....
Average	10.30	1.51	[1] 2.73	.....	7.89	.....	[1] 4.70	5.04
L. A. Brown, Kentucky	10.31	1.50	2.88	.....	8.60	.09	5.11	.....
	10.31	1.52	2.83	.....	8.65	.09	[1] 5.26	.....
	.....	1.53	2.76	.....	.....	.....	.....	.....
	.....	1.56	.....	.....	.....	.....	.....	.....
Average	10.31	1.53	[1] 2.78	.....	8.62	.09	[1] 5.19	5.27
J. S. McHargue, Kentucky	10.34	1.53	2.56	.....	.....	.10	5.00	.....
	10.30	1.58	2.62	.....	.....	.08	5.02	.....
	.....	1.51	2.62	.....	.....	.....	4.96	.....
	.....	1.51	2.62	.....	.....	.....	5.00	.....
	.....	1.55	2.56	.....	.....	.....	.....	.....
	.....	1.55	2.50	.....	.....	.....	.....	.....
Average	10.32	1.54	2.58	.....	.....	.09	5.00	5.11
General average	10.36	1.53	2.52	.....	.....	.11	5.07	5.06

SAMPLE No. 2.

Geo. P. Gray, California	5.10	0.83	1.42	3.76	3.81	0.06	[1] 2.63	2.81
S. D. Averitt, Kentucky	5.09	.83	1.37	3.66	3.65	.07	2.79	.....
	5.07	.82	1.40	3.73	3.67	.06	2.78	.....
	5.10	.82	1.40	.....	3.61	.06	2.82	.....
	5.12	.83	1.39	.....	3.70	.....	.....	.....
	.....	.82	1.39	.....	3.73	.....	.....	.....
Average	5.10	.83	1.39	3.70	3.67	.06	2.80	2.79
Wm. C. Marti, Michigan	5.35	.84	1.24	3.96	3.97	.05	2.51	.....
	5.22	.83	1.28	3.99	4.10	.08	2.60	.....
	.....	.81	1.31	.....	.....	.....	.....	.....
	.....	.80	1.25	.....	.....	.....	.....	.....
Average	[1] 5.29	.82	[1] 1.27	[1] 3.98	4.04	.07	[1] 2.56	[1] 2.66

[1] Not included in the general average

Cooperative results on lime-sulphur solutions, 1912—Continued.

SAMPLE NO. 2—Continued.

Analyst.	Total sulphur.	onosulphur equivalent.	Thiosulphate sulphur.	Sulphid weighed as barium sulphate.	Sulphur weighed as sulphur.	Sulphate and sulphite sulphur.	Calcium oxid.	
							Determined.	Calculated.
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
W. B. Pope, Washington, D. C.	5.16	0.66	1.62	.....	3.63	.....	2.49	.....
	5.16	.65	1.62	.....	3.39	Trace.	2.53	.....
	5.16	.66	1.66	.....	3.53	.....	.....	.....
Average	5.16	[1].66	[1]1.63	.....	3.52	.....	[1]2.51	[1]2.59
C. C. McDonnell, Washington, D. C.	5.04	.64	1.88	.....	3.45	.....	2.50	.....
	5.10	.65	1.82	.....	3.34	.....	.....	.....
	.....	.66	1.80	.....	.....	.....	.....	.....
Average	5.07	[1].65	[1]1.83	.....	3.40	.....	.....	2.75
L. A. Brown, Kentucky	5.14	.83	1.40	.....	4.33	.06	2.82	.....
	5.12	.83	1.40	.....	4.33	.06	2.86	.....
Average	5.13	.83	1.40	.....	4.33	.06	2.84	2.80
J. S. McHargue, Kentucky	.....	.85	1.27	.....	.....	.05	2.70	.....
	.....	.82	1.35	.....	.....	.06	2.70	.....
	.....	.82	1.31	.....	.....	.....	.....	.....
	.....	.82	1.37	.....	.....	.....	.....	.....
Average	.....	.83	1.33	.....	.....	.06	2.70	2.72
General average	5.11	.83	1.39	.....	.....	.06	2.78	2.80

[1] Not included in the general average.

The results of seven chemists on the two samples of lime-sulphur solution are given in this table. It will be noted that many determinations have been omitted from the general average of results because the referee was unwilling to let results that are manifestly erroneous vitiate a method. In justice to the writer it should be stated that those analysts whose results were omitted were afforded an opportunity to repeat their determinations. No general averages are given for sulphid sulphur weighed either as barium sulphate or as sulphur, as only three chemists report results on the former, which was the method to be tested. Two of the three analysts, however, obtained good results by weighing as barium sulphate and one by weighing the sulphur directly. It was suggested that this method of weighing the sulphur directly be tried, as it was short and easy, but it was not mentioned in the original instructions and does not appear in the recommendations. The method of weighing the sulphur on a filter in the manner suggested is open to criticism and is not good theoretically, but in the writer's hands it has given excellent results and he believes it is worthy of further consideration.

In the case of four of the seven analysts the results for lime on sample No. 1 show almost perfect agreement between the determined value (weighed as calcium oxid) and the calculated value from the iodin titration and from the sulphate sulphur. This is as it should be, and if there is a difference of more than 0.10 per cent the analysis should be repeated.

In sample No. 2 the same is true of the results of three of the seven analysts.

In the general averages of No. 1 the ratio of the sulphid sulphur to the monosulphur equivalent=7.72÷1.53=5.05, which shows that the sulphid sulphur is present as pentasulphid (CaS_5), while in sample No. 2 the ratio is 4.4, which indicates that about 44 per cent of the sulphid sulphur is pentasulphid and the remainder tetrasulphid (CaS_4). The writer can not understand some of the results reported for lime. It seems to him that the determination of lime was the simplest proposition submitted, and he was relying upon this as a check upon the work. In sample No. 1, with about

5 per cent lime, the maximum is 5.26 per cent, the minimum 4.54 per cent—a difference of 0.7 per cent. The impossibility of such figures being correct can be easily shown.

LEAD ARSENATE.

In accordance with a suggestion made last year the association instructed the referee to investigate the time of standing for the solution of soluble arsenic and the effect of temperature thereon. After having consulted several prominent fruit growers relative to the prevailing customs in the practical use of the lead arsenate, it was found that from 2 to 4 pounds of the paste in 50 gallons of water was the usual concentration; that it was always applied in from two to six hours after mixing, and that no attention was paid to the temperature, which is probably 60° to 70° F. The writer decided that the digestion should be made under as nearly as possible the same conditions as obtain in its practical use. It is not at all probable that any considerable solution of the arsenic or hydrolyzation takes place after its application.

Curry and Smith of the New Hampshire station have shown that with 18 hours' stirring at 20° C. there was no further solution with 2 grams in 500 cc of water, but that a correction proportional to the amount of water used in the digestion had to be applied for the solubility of lead arsenate. According to their findings, the solubility of lead arsenate under the actual conditions of its use would be about one-fifth that under the conditions of the old method of digestion (1 gram in 1 liter of water). It was clearly shown by Curry and Smith that when the water-soluble arsenic, as shown by the provisional method, is corrected, for the solubility of lead arsenate under the same conditions, there is shown a relatively small amount of uncombined arsenic oxid, and that is what we are really seeking. It is hard to conceive of a method giving results so wide of the mark, and requiring so long to make a determination, remaining a provisional method.

It would seem that 1 gram in 200 cc of water, which is in close agreement with the amount of water used in field operations, and the methods and times of digestion called for in this year's work, which are probably as nearly those of field work as can be secured in the laboratory, should give nearer the correct figure for soluble arsenic than the abnormal quantity of water and the 10 days' standing of the present provisional method. At least it is less favorable to hydrolyzation, which renders the old method worthless (without correction) so far as determining the real character of the sample is concerned.

RESULTS OF 1912 WORK.

Only three chemists report any work on the sample of lead arsenate, and while more work would have been desirable enough was done to settle the following question: Will three hours' shaking in a mechanical shaker and two hours' standing give the same results as shaking every half hour during a working day and letting stand overnight before filtering? No comparison of the results obtained by the short digestions with the provisional method was attempted because, for the reasons stated above, they are not comparable. The writer is well aware of the fact that much difference in temperature would materially affect the solubility of both arsenic oxid and lead arsenate, but as temperature is not considered in practical work its injection as a factor in the determination seems to be entirely uncalled for.

The results of the three chemists are given in the table following.

Water-soluble arsenic in lead arsenate paste.

[Water-free basis.]

Analyst.	Method I.	Method II.	Analyst.	Method I.	Method II.
	Per cent.	*Per cent.*		*Per cent.*	*Per cent.*
Geo. P. Gray, California...	0.35	[1] 0.12	Wm. C. Marti, Michigan...	[1] 0.41	0.27
				.33	.31
S. D. Averitt, Kentucky..	.25	.27		.33	.33
	.29	.25		[1] .54	.27
	.29	.25		.33	.33
	.23	.23		.37	[1] .42
Average.....	.27	.25	Average.....	.39	.33
			General average.....	.31	.28

[1] Not included in the general average.

It appears from the above table that with the exception of two or three determinations the results obtained by the two methods of digestion agree fairly well in the hands of the same analyst, and with one abnormally low and one high determination, besides two others that are probably questionable, left out of the general average, the concordance is as good as could be expected in this character of work. The referee feels satisfied that either method of digestion may be employed with very slight differences in the results.

Before closing his report the writer would like to call the association's attention to two methods under insecticides that should receive some attention in the near future. The first is the determination of arsenous oxid in Paris green. Mr. C. C. Hedges, of Cornell University (J. Ind. Eng. Chem., 1909, *1* : 208) has published a method which requires only about one-fourth the time required by the methods now used, which is quite a consideration to a busy analyst. The second is the Kissling method for nicotin in tobacco extracts, which in our laboratory has not proved accurate, and s rather tedious. On the other hand, Lloyd's method is direct and in our laboratory has given good results.

The referee wishes to thank the various chemists who have assisted him in this work and to state to the association that his work has been solely in the interest of good workable methods.

Recommendations.

It is recommended—

(1) That recommendiaton 1 of last year, which was adopted at that time and referred to this meeting for final action, be adopted (Bul. 137, p. 41, and Cir. 66, p. 2). This recommendation refers to the chromate method for total lead oxid in lead arsenate.

(2) That recommendations 3, 4, 5, and 6 of last year (Cir. 90, pp. 6–7), relating to the analysis of lime-sulphur solutions, be not adopted.

(3) That recommendation 12 of last year, which was adopted and referred to this meeting for final action, be adopted (Bul. 137, p. 47, (7)). This recommendation refers to mixing the sample and the determination of moisture, free acetic acid, and free ammonia in lead arsenate.

(4) That the following methods for the analysis of lime-sulphur solutions, which are accurate, direct, and eminently practical, be made official methods of this association:

(a) *Total sulphur.*—Weigh accurately 5 to 10 grams of the solution, depending upon the concentration. Make to 100 cc with carbon dioxid free water and use 10 cc aliquots for each determination. (In the case of concentrates 5 grams should be made to 200 cc and 10 cc aliquots used.) Transfer the aliquots to a wide-neck Erlenmeyer flask. Add 3 grams of sodium peroxid. Cover immediately with a watch glass and heat on steam bath 5 or 6 minutes after shaking it thoroughly. Dilute a little and add 10 cc concentrated hydrochloric acid. Make to about 140 cc;

boil a few minutes to drive off the dissolved gases, then add, drop by drop, at the rate of 5 cc per minute, 10 cc of a 10 per cent solution of barium chlorid. The solution must not be shaken during the addition of the barium chlorid. Set aside to cool. The precipitation may be made with equal accuracy in the cold, provided 5 per cent of barium chlorid solution is used instead of 10 per cent. If precipitated in the cold it should stand overnight before filtering. (See Otto Folin's article, J. Biol. Chem., 1905, 1: 131.) The precipitated barium sulphate should be heated in a closed crucible at very low heat until the filter is thoroughly charred. Then partially remove the lid and continue ignition gradually until constant weight with the full heat of the Bunsen flame. Calculate sulphur from weight of barium sulphate.

(b) *Thiosulphate sulphur.*—Dilute 10 cc of solution prepared for total sulphur to about 30 cc in a wide-neck Erlenmeyer flask and titrate with tenth-normal iodin until the yellow color of the polysulphids just disappears. This point is easily determined provided the flask is surrounded by white surfaces over a white plate. Titrate very carefully near the disappearance of the color, with thorough shaking. Note the volume of iodin used and continue the above titration, letting the iodin act as its own indicator until a small drop produces a slight permanent coloration. The additional iodin represents thiosulphate sulphur according to the following reaction: $2\ CaS_2O_3 + I_2 = CaI_2 + CaS_4O_6$ (1 cc N/10 I=0.0064 S).

The iodin used to discharge the color of the polysulphids determines the monosulphur equivalent as follows: $I_2 = S$ (1 cc N/10 I=0.0016 S).

(c) *Sulphate and sulphite sulphur.*—To the filtrate with one washing from the thiosulphate determination add several drops of hydrochloric acid and precipitate in the cold with about 5 cc of 5 per cent barium chlorid. Let stand overnight in the cold; from the weight of barium sulphate calculate the sulphate and sulphite (now sulphate) sulphur.

(d) *Sulphid sulphur.*—Direct determination: The filtered and washed sulphur from the thiosulphate determination is placed with the filter paper in a wide-neck Erlenmeyer flask and the sulphur dissolved in 15 cc (1 to 3) sodium hydroxid (do not use potassium hydroxid), heating on steam or water bath until the sulphur dissolves (do not boil). It requires from 1 to 1½ hours on steam bath to dissolve the sulphur. Keep the flask covered and shake gently a few times during the digestion to get the sulphur off the sides. Oxidize and complete the determination as in total sulphur.

NOTE.—A blank must be run on the sodium hydroxid used and correction made therefor.

The difference between the sum of the thiosulphate and the sulphate sulphur and the total sulphur is sulphid sulphur.

(e) *Total lime (CaO) in solution.*—Oxidize 10 cc of solution prepared for total sulphur, as for total sulphur, make acid in the same way, dilute to about 50 cc, and boil a few minutes to drive off dissolved gases; make faintly alkaline with ammonium hydroxid, and if a precipitate occurs filter and wash. Precipitate with ammonium oxalate in the usual way and weigh calcium oxid.

(5) That the following method of digestion and determination of water-soluble arsenic in lead arsenate be made a provisional method of the association:

Water-soluble arsenic.—Weigh to 0.01 gram about 2 grams of paste. Place in a tightly stoppered bottle with 200 cc carbon dioxid free water per gram. Put in a mechanical shaker and shake for 3 hours. Let stand 2 hours and filter, or, instead of the mechanical shaker, shake every half hour or as often as possible during a working day. Let stand overnight and filter. Use 200 cc of the clear filtrate for the determination. Add 0.5 cc sulphuric acid and proceed as directed under water-soluble arsenic oxid, page 240, Bulletin 107, Revised, Bureau of Chemistry. It is important that the solution shall be perfectly clear and the titrations very carefully done. Make correction for amount of iodin to produce the same color, using same chemicals and volumes.

COMPOSITION AND METHODS OF ANALYSIS OF LIME-SULPHUR SOLUTION.

By C. C. McDonnell.

Since some doubt has been raised as to the accuracy of the methods proposed for the analysis of lime-sulphur solutions which were adopted provisionally by the association at its last meeting,[1] I wish to present the results of some further work which has been done in the Insecticide and Fungicide Laboratory of the Bureau of Chemistry on this subject since that time. When, as referee on insecticides, I presented these methods and recommended them for adoption by the association last year, I felt from the work which we had done on the methods and that presented by others who had cooperated with us that the results obtained were as nearly correct as one could reasonably desire on solutions of such a nature and more satisfactory as regards both manipulation and accuracy than any methods which had previously been proposed. These methods are essentially those given in Sutton's Volumetric Analysis and other standard works on quantitative analysis for the examination of alkali sulphid solutions, and mixtures of alkali sulphids, thiosulphates, sulphates, etc.

When the method for the determination of sulphid and thiosulphate sulphur in lime-sulphur solutions by direct titration with standard iodin solution was first published,[2] we did some work on it and came to the conclusion that the method was inaccurate and not a suitable one for the examination of lime-sulphur solutions in official work. The principal point in the contention of the present referee, which he holds indicates the incorrectness of the zinc precipitation method, is the fact that in some cases the results show by calculation a polysulphid greater than the pentasulphid, which, according to the literature, has not been proved to exist. In the tabulated results of analyses of lime-sulphur solutions by the two methods, we find more cases where a higher sulphid than the pentasulphid is indicated by the direct iodin titration method than by the zinc precipitation method. In Michigan Technical Bulletin No. 6, in which the method first appeared, the results of analyses of 29 lime-sulphur solutions, prepared and treated in various ways, are reported. In five of these the ratio of the total sulphid sulphur to the "monosulphur equivalent" value is greater than 5 to 1, the highest being 5.11 to 1. The samples sent out by the referee this year showed by three different analysts employing the titration method a ratio of total sulphid sulphur to the "monosulphur equivalent" determination greater than 5 to 1, the greatest being 5.38 to 1. A sample of commercial lime-sulphur solution purchased on the market showed by this method a ratio of 5.16 to 1.

In reference to results obtained by the methods which were adopted provisionally last year, I have tabulated the analyses of 33 samples of lime-sulphur solutions prepared in various ways, some of them made in the laboratory and some commercial solutions obtained on the market, containing from 2.5 to 30 per cent total sulphur. Total calcium was also determined and the ratio of calcium to polysulphid sulphur calculated in the following manner:

The amount of calcium required to combine with sulphate and thiosulphate sulphur was deducted from the total calcium. Then the sulphur equivalent of the calcium remaining was calculated and this divided into the total polysulphid sulphur determined. In only one case was the ratio of polysulphid sulphur to calcium greater than 5 to 1, the ratio being 5.08 to 1. The other 32 ranged between 5 and 3.5 to 1. All of the ratios below 4 to 1 were on dilute solutions prepared in the laboratory by mixing together lime and sulphur in about equal proportions and boiling in water, no effort being made to prepare concentrated solutions or the higher polysulphids. These results are given in Table 1.

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[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 152, pp. 70, 85.

[2] Michigan Agricultural Experiment Station Technical Bul. 6.

TABLE 1.—*Ratio of calcium to polysulphid sulphur in dilute lime-sulphur solutions.*

Total sulphur.[1]	Polysulphid sulphur.	Thiosulphate sulphur.	Sulphate sulphur.	Total calcium.	Calcium to combine with sulphate and thiosulphate sulphur.	Calcium remaining.	Ratio calcium to polysulphid sulphur.
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
3.22	2.59	0.63	Trace.	1.22	0.39	0.83	1–3.90
3.67	2.91	.76	Trace.	1.39	.48	.91	1–4.00
3.76	2.91	.84	0.01	1.52	.54	.98	1–3.71
3.79	2.92	.86	.01	1.37	.55	.82	1–4.45
2.55	2.05	.48	.02	.95	.33	.62	1–4.13
3.19	2.54	.63	.02	1.23	.42	.81	1–3.92
3.24	2.54	.68	.02	1.30	.45	.85	1–3.74
3.23	2.52	.69	.02	1.36	.46	.90	1–3.50
3.26	2.50	.74	.02	1.36	.49	.87	1–3.60
5.36	4.26	1.08	.02	2.11	.70	1.41	1–3.78
5.35	4.34	.99	.02	1.99	.64	1.35	1–4.02
3.23	2.53	.69	.01	1.30	.44	.86	1–3.68
3.21	2.53	.67	.01	1.27	.43	.84	1–3.76
3.20	2.53	.66	.01	1.24	.42	.82	1–3.85

[1] Total sulphur=polysulphid sulphur +thiosulphate sulphur +sulphate sulphur.

In Table 2 are given the results obtained on concentrated commercial solutions and those prepared in the laboratory by boiling together, in a small amount of water, lime with an excessive amount of sulphur.

TABLE 2.—*Ratio of calcium to polysulphid sulphur in concentrated lime-sulphur solutions.*

Total sulphur.	Polysulphid sulphur.	Thiosulphate sulphur.	Sulphate sulphur.	Total calcium.	Calcium to combine with thiosulphate and sulphate sulphur.	Calcium remaining.	Ratio calcium to polysulphid sulphur.
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
27.01	25.94	0.89	0.22	7.95	0.83	7.12	1–4.55
26.70	26.12	.63	.24	7.71	.69	7.02	1–4.65
26.56	25.96	.61	.12	7.68	.53	7.15	1–4.54
26.52	26.06	.52	.09	7.84	.44	7.40	1–4.40
24.61	22.38	2.21	.05	8.09	1.44	6.65	1–4.21
22.44	20.05	2.17	.06	6.96	1.43	5.53	1–4.53
21.86	20.88	.96	.08	6.69	.70	5.99	1–4.36
22.67	21.07	1.83	.06	6.49	1.22	5.27	1–5.00
22.17	20.86	1.04	.00	6.29	.65	5.64	1–4.62
23.20	21.36	1.50	.00	6.55	.94	5.61	1–4.76
24.08	23.61	.71	.00	6.99	.44	6.55	1–4.51
23.78	22.54	.97	.00	6.94	.61	6.33	1–4.45
24.87	24.00	.85	.00	7.16	.53	6.63	1–4.53
25.24	24.39	1.07	.00	7.04	.67	6.37	1–4.79
25.42	25.26	.20	.05	7.54	.19	7.35	1–4.30
24.59	23.00	1.16	.05	7.09	.79	6.30	1–4.56
24.81	23.54	.97	.00	7.02	.61	6.41	1–4.59
24.25	22.12	1.91	.00	6.64	1.20	5.44	1–5.08
30.78	29.37	1.28	.05	8.54	86	7.68	1–4.78

These results indicate that there is no calcium polysulphid in solution higher than the pentasulphid, CaS_5.

In this connection Divers and Shimidzu [1] state:

We find that although more than 5 atoms of sulphur to 1 of calcium may enter into solution during the reaction of sulphur upon the hydrosulphid (or the hydroxid), still when a pure pentasulphid solution has once cooled and deposited, as it does, the excess over 5 atoms, it can not again be made to take up a perceptible quantity of additional sulphur by boiling this with it.

[1] J. Chem. Soc., 1884, **45**: 283.

The referee points out that in the direct iodin titration method the calcium calculated from the monosulphid equivalent titration, the thiosulphate titration and sulphate determined, agrees with the calcium determined directly, thus apparently proving the correctness of the direct iodin titration method. This, however, is misleading, in consequence of the fact that as the dilution is increased the monosulphid equivalent value decreases and the thiosulphate value increases, and when calculated to calcium the two errors nearly counterbalance each other unless the dilution is considerable. Any conclusions deduced from such calculations are therefore erroneous.

Effect of Dilution on Monosulphid Value and Thiosulphate.

(a) 10 cc (14.06 grams) of a concentrated lime-sulphur solution prepared in the laboratory and containing nearly 31 per cent total sulphur were made up to 300 cc with distilled water which had previously been boiled.

(b) 5.27 grams of a sample of commercial lime-sulphur containing about 24 per cent total sulphur were made up to 200 cc.

To 10 cc aliquots of these solutions varying amounts of water were added, and the following results were obtained upon titration with iodin solution:

Solution.	Aliquot.	Made up to—	Monosulphid equivalent.	Sulphur as thiosulphate.	Solution.	Aliquot.	Made up to—	Monosulphid equivalent.	Sulphur as thiosulphate.
	cc.	*cc.*	*Per cent.*	*Per cent.*		*cc.*	*cc.*	*Per cent.*	*Per cent.*
(a)	10	20	6.00	1.73	(b)	10	50	4.59	1.34
	10	60	5.94	2.00		10	100	4.40	1.75
	10	250	5.70	3.06		10	200	4.36	2.30

These results show that the greater the dilution the less monosulphid equivalent is indicated and higher thiosulphate, the latter showing almost twice as much when diluted to 200 or 250 cc before titrating. The more concentrated the solution when the titration is made the more nearly the results agree with those obtained where the sulphids are first removed as zinc sulphid. An explanation will be given for this subsequently. It is not due to any actual increase in the thiosulphate.

Effect of Dilution on the Thiosulphate as Determined by the Provisional Method.

Solutions were prepared of two samples of lime-sulphur and treated as follows:

Ten cc and 25 cc aliquots of these solutions were diluted to 50, 100, and 250 cc, then ammoniacal zinc chlorid was added in slight excess and made up to 100, 200, and 500 cc. These solutions were filtered off, 50, 100, and 250 cc aliquots taken, carefully neutralized with dilute hydrochloric acid, and titrated with standard iodin solution. The results obtained are as follows:

Aliquot.	Diluted to—	Thiosulphate sulphur.		Aliquot.	Diluted to—	Thiosulphate sulphur.	
		Sample 3.	Sample 4.			Sample 3.	Sample 4.
cc.	*cc.*	*Per cent.*	*Per cent.*	*cc.*	*cc.*	*Per cent.*	*Per cent.*
10	50	1.09	3.98	25	50	1.08	3.83
10	100	.99	3.88	25	100	1.07	3.85
10	250	1.04	3.80	25	250	1.04	3.80

We see that by this method no more thiosulphate is obtained after diluting the solutions than when working on concentrated solutions, showing that simply diluting lime-sulphur solutions with water does not increase the thiosulphate.

Free Calcium Hydroxid in Lime-Sulphur Solutions.

While there is probably no free calcium hydroxid in freshly prepared lime-sulphur solutions, if an excess of sulphur was present during the digestion, there is nothing to prevent its being added thereafter and placed on the market in this condition. Also, if lime-sulphur solution is diluted sufficiently with water, calcium hydroxid is formed and if the hydrogen sulphid produced at the same time is removed from the solution, calcium hydroxid will remain as such. Old or partially decomposed lime-sulphur solutions therefore may contain calcium hydroxid. The direct iodin titration method would not be applicable at all to such solutions. A lime-sulphur solution which showed by direct titration monosulphid equivalent 5.26 per cent and thiosulphate sulphur 3.54 per cent, gave, after the addition of 15 cc clear lime water, monosulphid equivalent 6.54 per cent and thiosulphate sulphur 4.12 per cent. Iodin is used up by the calcium hydroxid and both end points are difficult to read.

There is no way of knowing absolutely the amount of thiosulphate in a lime-sulphur solution. We know that calcium thiosulphate as well as zinc thiosulphate is extremely soluble in water. It did not seem at all probable in precipitating the polysulphid with zinc that any appreciable amount of thiosulphate would be occluded in the precipitate. The possibility, however, of this being a source of error was not overlooked. In making up the samples for the work last year [1] a solution of lime-sulphur was prepared and divided into two parts, to one of which was added sodium thiosulphate in sufficient amount to increase the sulphur as thiosulphate 2.02 per cent. The results submitted by eight analysts agreed well and the average of all determinations of sulphur as thiosulphate showed 1.98 per cent more in the sample to which the sodium thiosulphate had been added, or 0.04 per cent less than the amount actually added. I have since prepared lime-sulphur solution containing greatly varying amounts of sodium thiosulphate up to 50 times the amount occurring in the sample, and recovered practically the amount added in every case.

Causes of the Inaccuracy of the Iodin Direct Titration Method.

The referee assumes that lime-sulphur solutions contain only calcium polysulphids, calcium thiosulphate, and calcium sulphate, and that no change is produced in the solution on diluting with water. This, however, is not the case as has been shown by Divers & Shimidzu,[2] upon whose work most of the following statements are based.

Calcium pentasulphid when treated with water produces calcium hydroxid, free sulphur, and hydrogen sulphid.

$$CaS_5+2H_2O=Ca(OH)_2+4S+H_2S.$$

Some of the hydrogen sulphid may escape and some combines with calcium hydroxid, forming calcium sulphydrate.

$$Ca(OH)_2+2H_2S=Ca(SH)_2+2H_2O.$$

The calcium sulphydrate reacts with water, forming calcium hydroxy-hydrosulphid.

$$Ca(SH)_2+H_2O=Ca(SH)(OH)+H_2S.$$

The calcium hydroxy-hydrosulphid may react further with water, forming calcium hydroxid.

$$Ca(SH)(OH)+H_2O=Ca(OH)_2+H_2S.$$

These decompositions proceed only when the hydrogen sulphid produced can escape or become diluted. Anyone may observe that on diluting a lime-sulphur solution with a large quantity of water a milky appearance is produced almost immediately from the precipitation of free sulphur. The fact that hydrogen sulphid is given off can readily be shown by putting some lime-sulphur solution in a flask, adding about an equal quantity of water, and suspending above the solution a strip

[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 152, p. 70.
[2] J. Chem. Soc., 1884, *45:* 270–291.

of moistened lead acetate paper, which after a time will turn black. It has frequently been noticed that upon opening a can or barrel of commercial lime-sulphur solution a considerable pressure has developed from the accumulation of hydrogen sulphid.

On diluting lime-sulphur with water, therefore, we may have in the solution, in addition to the calcium polysulphid and thiosulphate originally present, hydrogen sulphid, calcium hydroxy-hydrosulphid, and calcium hydrosulphid, all of which compounds are readily soluble in water, forming clear solutions. Diluting the solution, however, does not produce any thiosulphate. On titrating with iodin, the yellow color of the polysulphid will have disappeared before these colorless sulphur compounds have been attacked, and they will then be titrated and calculated as thiosulphate. The greater the dilution, the greater will be the decomposition of the polysulphid and the more calcium hydrosulphid and hydroxy-hydrosulphid will be formed; in fact, if the dilution be sufficiently great, all of the polysulphid will be decomposed. The sulphur of calcium hydrosulphid and hydroxy-hydrosulphid is precipitated by zinc salts and therefore by the zinc precipitation method is determined as sulphid and does not interfere with the thiosulphate determination. These facts explain why the monosulphid equivalent determination by the direct iodin titration method is too low and the thiosulphate determination too high; also why on increasing the dilution with water there is shown an apparent increase in thiosulphate.

The more concentrated the solution when the titration is made, the more nearly the results agree with the zinc precipitation method.

In addition to this error introduced by dilution, there is also a chance for considerable error owing to the difficulty of determining the end point in the titration. The referee recommends that in the case of "concentrates" 5 grams be made to 200 cc and 10 cc aliquots of this solution used for the titration. Practically all of the lime-sulphur solutions obtained on the market are concentrated solutions and would therefore come under this head. By such a dilution as this we should be working upon aliquots of 0.4 gram original material. In the thiosulphate titration 0.1 cc of tenth-normal iodin solution is equivalent to 0.64 mg of sulphur. In the titration for monosulphur equivalent it is impossible to determine the end point with any degree of certainty closer than 2 drops, or 0.1 cc of tenth-normal solution, and in the thiosulphate titration the difference can easily be 2 drops, or 0.1 cc. Thus the total error in the titration alone, where conducted under exactly the same conditions of dilution, etc., may easily be 0.2 cc tenth-normal iodin solution, equivalent to 1.28 mg of sulphur, or 0.32 per cent. The natural tendency in the titration is always to get too low monosulphid equivalent and too high thiosulphate.

The average thiosulphate determination made on 16 samples by the direct iodin titration method gave 2.61 per cent thiosulphate sulphur and by the zinc precipitation method 2.07 per cent thiosulphate sulphur; or 0.54 per cent lower by the second method. One-half of this difference can be accounted for by errors in the observation of the end point alone. If the direct iodin titration method had been run on more dilute solutions the average difference in the results would have been much greater than here shown.

In regard to rapidity, the methods for the determination of the total sulphur are practically the same in both. The determination of thiosulphate by the method of removing the polysulphid sulphur by ammoniacal zinc chlorid and then titrating the thiosulphate by iodin requires very little more time than the direct titration method. For the determination of polysulphid sulphur the direct weighing of the sulphur is inaccurate, so that it is necessary to let the solution stand for some time after the titration in order that the sulphur may collect, then filter, dissolve the sulphur in sodium or potassium hydroxid, oxidize to sulphate, and precipitate as barium sulphate. This operation requires more time than the method of precipitation as zinc polysulphid.

Objection has been raised to the zinc precipitation method on account of the difficulty of filtering and handling the zinc polysulphid precipitate. We have not found this a valid objection, as the precipitate settles readily, filters quickly, and is reasonably easy to wash. No oxidation takes place during the washing, at least no soluble sulphur compound is formed that is washed through. I have repeatedly tested this by examining the filtrate. The zinc polysulphid precipitate does not carry down any thiosulphate if the zinc chlorid solution used for the precipitation is strongly ammoniacal.

As a result of this additional work I am more strongly of the opinion than ever that the methods as adopted provisionally by this association give more accurate results and are more satisfactory than the direct iodin titration method.

REPORT ON WATERS.

By W. W. Skinner, *Referee.*

After consultation with the associate referee, it was decided that the cooperative work on waters should be varied from the usual procedure of the last few years of sending out a sample for complete analysis. Therefore, our efforts this year have been restricted to some of those determinations which had been found in previous reports to cause the greatest difficulty in obtaining satisfactory and concordant results. The work has been confined to a study of the phenol sulphonic acid method and the reduction method of determining nitrogen in the form of nitrates, a further study of the method proposed last year for the determination of strontium, and a study of the Haywood colorimetric method developed for the determination of small quantities of bromin and iodin. Fifteen chemists agreed to take part in the cooperative work, and samples were sent to each, but, owing no doubt to the unusually early date for the meeting of the association this year, only nine reports were received in time for consideration in the report of the referee. Three samples were prepared for the work by adding known quantities of certain salts to distilled water, the resulting solutions being of such strength that the aliquot to be used in each case represented approximately the amount of the desired constituent that might be expected to give a fair test of the method as employed under ordinary laboratory working conditions, and also fairly represented the quantities usually found in the amounts of water generally prescribed for the several determinations. The methods and directions in detail sent to each collaborator were as follows:

Directions for Cooperative Work on Water, 1912.

PHENOL SULPHONIC ACID METHOD FOR NITRATES.

Add standard silver sulphate free from nitrate (4.3969 grams per liter; 1 cc equals 1 mg of chlorin), precipitating all but about 0.5 mg of the chlorin. Heat to boiling, allow to settle, or add a little alumina cream, filter, and wash with small amounts of hot water. Evaporate the filtrate to dryness in porcelain; when cold add 2 cc of phenol disulphonic acid reagent,[1] rubbing with a glass rod to insure intimate contact. Dilute with distilled water and add slowly ammonium hydroxid until the maximum color is developed. Transfer to a colorimetric cylinder, filtering if necessary, and compare with a standard potassium nitrate solution [2] (containing 0.01 mg of nitrogen as nitrate in 1 cc) which has been treated in like manner with phenol disulphonic acid reagent. Compare in the usual manner and record as nitrogen in the form of nitrates.

REDUCTION METHOD FOR NITRATES.

Reagents.—(1) Sodium or potassium hydroxid solution: Dissolve 250 grams of the purest hydroxid obtainable in 1.25 liters of distilled water and boil down to 1 liter.

[1] Prepared by dissolving 25 grams pure white phenol in 150 cc c. p. sulphuric acid, concentrated, and 75 cc fuming sulphuric acid 13 per cent SO_3 and heating at 100° C. for two hours.

[2] A portion of the standard potassium nitrate solution may be evaporated, the residue treated with phenol disulphonic acid, taken up with water, and made up to a definite volume. This solution will keep, and standards for comparison may be prepared by adding ammonia to measured volumes of it.

(2) Aluminum foil: Use strips about 5 cm long, 0.012 mm thick, and of such a width that each strip weighs about 0.35 gram.

Procedure.—Put 10 cc of the sample in a Nessler tube and make up to exactly 50 cc; add 5 cc of sodium hydroxid solution and a strip of aluminum foil. Close the mouth of the Nessler tube with a rubber stopper carrying a U-shaped glass tube of such length that the outlet can be placed in a second test tube containing a small amount of dilute hydrochloric acid which serves as a trap to catch any ammonia which may escape. Allow to stand at room temperature for 12 hours or more until reduction is complete; transfer contents of tube to a Kjeldahl flask and distill; cool the distillates and nesslerize in the usual way. Nesslerize the solution in traps also.

METHOD FOR CALCIUM AND STRONTIUM.

Dilute 10 cc of Solution 3 to about 150 or 200 cc and to this solution add from 1 to 2 grams of oxalic acid and sufficient hydrochloric acid to clear the solution. Heat to boiling and neutralize with ammonium hydroxid, stirring constantly. Add ammonium hydroxid in slight excess and allow to stand for 3 hours, preferably in a warm place; filter off the supernatant liquid and wash precipitate by decantation once or twice with cold water. Dissolve precipitate with hydrochloric acid, dilute to from 100 to 200 cc; add a little oxalic acid and precipitate as before. After standing 3 hours, filter, wash with cold water,[1] dry, ignite, and blast, and weigh as calcium and strontium oxids.

Dissolve the above oxids with dilute nitric acid and test with the spectroscope for strontium. If strontium is found, transfer the nitric acid solution to a small Erlenmeyer flask and evaporate to complete dryness on the steam bath. Heat at 150° to 160° C. for 1 or 2 hours until perfectly dry; cool, add from 3 to 5 cc of a mixture of equal parts of absolute alcohol and ether to dissolve the calcium nitrate; cork the flask and allow to stand for 12 hours with occasional shaking. Filter, wash with alcohol ether mixture until a few drops of the filtrate evaporated on platinum foil or watch glass leave no residue.

Dry the paper and precipitate. Dissolve the strontium nitrate with a few cubic centimeters of hot water. Add a few drops of sulphuric acid, then a volume of alcohol equal to the volume of the solution; allow to stand 12 hours. Filter, ignite, weigh as strontium sulphate, and calculate to strontium. Test spectroscopically for absence of calcium. Subtract from the total oxids of strontium and calcium the strontium oxid equivalent to the strontium sulphate found. The difference is calcium oxid. Calculate to calcium. As a check on the calcium oxid, evaporate to dryness the filtrate from the strontium nitrate; dissolve the calcium nitrate in water, precipitate as oxalate, filter, wash, ignite, and weigh as calcium oxid.

HAYWOOD'S COLORIMETRIC METHOD FOR IODIN AND BROMIN.

To 10 cc of Solution 3, diluted to about 100 cc, add sodium carbonate in slight excess of the amount necessary to precipitate calcium and strontium and evaporate to dryness. Boil the residue with distilled water; transfer to filter, and thoroughly wash with hot water. Evaporate the alkaline filtrate to dryness, add 2 or 3 cc of water to dissolve the residue and enough absolute alcohol to make the percentage of alcohol about 90. Boil and filter and repeat the extraction of the residue with alcohol once or twice. Add 2 or 3 drops of sodium hydroxid to the combined alcohol filtrates and evaporate to dryness. Dissolve the residue with 2 or 3 cc of water and repeat the extraction with alcohol as before; add a drop of sodium hydroxid to the filtrate and evaporate to dryness.

Dissolve the residue in a little water, acidify with sulphuric acid (1 to 5), using 3 or 4 drops in excess, and transfer to a small flask. Add 4 drops of 2 per cent potassium nitrite (or sodium nitrite) solution and about 5 cc of carbon bisulphid freshly purified by distillation. Shake until all iodin is extracted; filter off the acid solution from the carbon bisulphid, wash the flask, filter, and contents with cold distilled water, and transfer the carbon bisulphid (containing the iodin in solution) to Nessler tubes, using approximately 5 cc of pure carbon bisulphid. In washing the filter make the contents of the tube up to definite volume, usually 12 or 15 cc, and match the color with that of other tubes containing known amounts of iodin dissolved in carbon bisulphid. Prepare the standard tubes by taking measured quantities of a solution of known potassium iodid content, acidifying with sulphuric acid (1 to 5), adding 3

[1] Usually two or three washings are sufficient. Owing to the solubility of strontium oxalate, excessive washings give low results.

or 4 drops of potassium nitrite solution, and extracting with carbon bisulphid as in the actual determinations.

Transfer to small flasks the sample and standards from which the iodin has been removed. To the standards add definite measured quantities of bromid solution of known strength, and to each of the flasks containing sample and standards add 5 cc of purified carbon bisulphid. Add saturated chlorin water, 1 cc at a time, shaking after each addition until all the bromin is set free.[1] Filter off the water solution from the carbon bisulphid through a moistened filter, wash the contents of the filter two or three times with water, and then transfer to a Nessler tube by means of about 1 cc of carbon bisulphid.

Repeat this extraction of the filtrate twice, using 3 cc of carbon bisulphid each time. The combined carbon bisulphid extracts usually amount to from 11.5 to 12 cc. Add enough carbon bisulphid to the tubes to bring them to a definite volume, usually 12 to 15 cc, and compare the sample with the standards. In some cases when working with the method near its upper limit the bromin is not all extracted by the amounts of carbon bisulphid recommended. If so, make one or two extra extractions with carbon bisulphid, transfer the extracts to another tube, and compare the color with some of the lower standards.

Discussion.

For some years past there has been a question as to the accuracy of the phenol sulphonic acid method as generally employed for the determination of nitrates. The cooperative work reported in previous years by both the former and the present referee gives ample evidence that the results obtained by this method leave much to be desired, and it was owing to the suggestion of one of the collaborators that it was decided to test the method in parallel with the reduction method, the latter as well as the phenol sulphonic acid method having been adopted as official by the American Public Health Association.

That chlorids seriously interfered with the accuracy of the phenol sulphonic method was early recognized by Mason, Leffman, Gill, and others, and attempts were made to overcome this difficulty by adding to the standards for comparison amounts of sodium chlorid equivalent to that known to exist in the aliquot of the water being examined. For waters of comparatively low chlorin content, this modification of the method, with much care in observing the strictest uniformity of technique with both samples and duplicates, will give fairly satisfactory results, but for samples high in chlorids the results are always too low. The origin of the use of silver sulphate in this method to remove the chlorin seems obscure, and while it was mentioned by Gill [2] in 1894, the modification did not attract the attention its merit warrants, and only in recent years has it been generally adopted. The use of silver sulphate with the substitution of potassium hydroxid for ammonium hydroxid, as suggested by Chamot,[3] was tested last year, and as a result of that work the use of potassium hydroxid was abandoned, the color obtained by ammonium hydroxid being found more uniform and no trouble being experienced from a precipitate when ammonium hydroxid is used. A precipitate may cause trouble when potassium hydroxid is used should there be a slight excess of silver sulphate present. The modified phenol sulphonic acid method as above described was recommended last year for adoption as a provisional method of the association.

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[1] Care must be taken not to add too much chlorin in excess of that necessary to set the bromin free, since a bromo-chlorid may be formed with an excess of the reagent, thus spoiling the color reaction.

[2] J. Amer. Chem. Soc. 1894, *16:* 122.

[3] J. Amer. Chem. Soc., 1909, *31:* 922; 1910, *32* 630; 1911, *33:* 366, 381.

Cooperative work on methods of water analysis.

[Results expressed in parts per million.]

Analyst.	Nitrogen in the form of nitrates, chlorids absent: Sample 1.		Nitrogen in the form of nitrates, 1,000 parts per million sodium chlorid present: Sample 2.		Calcium: Sample 3.	Strontium: Sample 3.	Bromin: Sample 3.	Iodin: Sample 3.
	Phenol sulphonic method.	Reduction method.	Phenol sulphonic method.	Reduction method.				
(1) S. D. Averitt, Lexington, Ky.	2.22 2.22	2.10 2.46	2.22 2.22	2.100 2.464	99.13 100.70	37.63 35.68		
	2.22	2.28	2.22	2.28	99.91	36.65		
(2) R. O. Baird and A. A. Jones, Stillwater, Okla.	2.53	2.48	1.55	2.00	165.31 164.82 [1]165.07	42.64 43.36 [1] 43.00	[1] 0.81	[1] 0.54
(3) H. P. Corson and C. S. Scholl, Urbana, Ill.	1.98	1.92	1.99	2.00	104.30	33.10	4.90	1.10
(4) C. N. Colver, Moscow, Idaho	2.57	[2] 1.67	2.32	[2] 1.61	95.92 96.42 96.35 96.23	39.50 38.90 39.20	2.67 2.67 2.67	1.00 1.00 1.00 1.00
(5) D. C. Dyer, Washington, D. C.	2.00	2.26	2.40	2.25	99.06	38.40 36.20 38.93	2.00	1.50
(6) C. M. Hargraves, Washington, D. C.	2.20	2.24	2.20	2.42	98.99	37.16	3.20	1.20
(7) M. E. Stover, Berkeley, Cal.	2.50	2.52	2.46	2.52	108.85	35.03	3.42	.99
(8) J. W. Sale, Washington, D. C.	2.10	1.90	2.20	2.20	99.96	36.49	2.62	1.45
(9) W. I. Watkins, Columbia, Mo.	2.16	1.76	1.87	1.80	96.53	35.16	2.43	.95
(10) Arao Itano, East Lansing, Mich.[3]	2.27	2.15	3.39	2.89	112.85	31.20	4.01	1.10
(11) C. C. Young, State Board of Health, Kansas [3]	2.20	2.20	2.00	2.20	98.00	40.00		
(12) J. P. Aumer and E. Van Alstine, Urbana, Ill.	1.8 1.8 2.0 1.87	3.3 3.8 3.55	2.0 1.9 1.9 1.93	2.6 2.6 2.6	101.06 102.64 105.00 101.49 97.06 101.14 101.40	28.91 27.62 35.87 32.34 31.72 32.34 31.47		
Average	2.25	2.11	2.13	2.12	100.48	36.46	3.03	1.17
Theory	2.1	2.1	2.3	2.3	100.1	41.4	3.36	1.53

[1] Omitted from average.
[2] Average of three.
[3] Received too late to be included in the report read at the meeting.

By reference to the table it will be found that the results by both the phenol sulphonic and reduction methods are very similar, the variation from theory being about the same by each of these methods. The allowable variation in matching the colors in the phenol sulphonic method is probably about 10 per cent of the reading when the standard solution contains as much as 0.10 mg of nitrogen to 100 cc. When the standard is less, say 0.05 mg to 100 cc, the variation should not be so great. It is therefore advisable to make the comparison of the unknown with the standard of

approximately the latter strength, diluting the unknown in order to obtain necessary data for calculation of the final result. By reference to the table it will be observed that the variation of the average from theory by the phenol sulphonic method is about 7.5 per cent. Analysts 2, 4, and 7, however, obtained results which vary much more than 10 per cent from theory. The results by the reduction method on the same sample yield an average which is 0.14 nearer theory than are the results by the phenol sulphonic method, or, stated differently, the average varies from theory by only 0.01, but it will be noted that the variation between the highest and lowest results reported is greater by the reduction than by the phenol sulphonic method, being 0.85 by the reduction method compared with 0.59 by the phenol sulphonic acid method. On Sample 2, which contains an equivalent of 1,000 parts per million of sodium chlorid, it will be noted that the average by the two methods is almost identical, being 2.13 and 2.12, respectively, while the variation between the highest and lowest results reported is identical, being 0.91 by each method.

The conclusions reached, therefore, are that the modified phenol sulphonic method using silver sulphate to remove chlorids is quite as accurate as the reduction method, even with samples very high in chlorids; that results can not be expected much closer than 10 per cent of theory by either method upon amounts such as were used in the work this year; and, as the phenol sulphonic method is easier of manipulation, it is to be preferred to the reduction method, regarding which one collaborator remarked, "There are too many chances for error in the reduction method for it to be safe in the hands of the average analyst. Besides, it takes too much time to make determinations."

The results reported for calcium were obtained incidentally to the determination of strontium and are tabulated because comparative results, even of such a well-known determination as calcium, are always interesting. The results for strontium show a variation between the highest and lowest accepted results of 6.1, the highest being 39.20 and the lowest 33.10. This is a variation from the average of 7.5 per cent for the highest and 9.2 per cent for the lowest, and is a variation similar to that shown for calcium. The variation of results on strontium from theory is inexplainable, as previous work has indicated that results approximating theory can be obtained by the proposed method. It would seem to indicate, however, that further work is necessary before adoption of the method as official.

The colorimetric method for bromin and iodin has yielded results that are not entirely satisfactory, but it should be remembered that it was originally proposed for quantities much smaller than those here present. The variation can in part be attributed to inexperience in working the method, but the difficulty is probably to be attributed very largely to the extraction of the iodids and bromids by alcohol, for it has been shown that when working on pure solutions, where extraction is unnecessary, the method yields fairly concordant and accurate results. It is advisable, therefore, that the extraction part of the method receive special attention from the referee next year.

Recommendations.

It is recommended—

(1) That the phenol sulphonic acid method for nitrogen in the form of nitrates be adopted as an official method.

(2) That the reduction method for nitrogen in the form of nitrates be adopted as an optional method.

(3) That the method for strontium be further studied.

(4) That the colorimetric method for iodin and bromin be further studied.

(5) That the methods for analysis of water as published in Bureau of Chemistry Circular No. 52 and modified in the Proceedings for 1911 be adopted as official, except the methods for strontium, bromin, and iodin.

REPORT OF COMMITTEE A ON RECOMMENDATIONS OF REFEREES.[1]

By J. P. Street, *Chairman.*

(Nitrogen, potash, phosphoric acid, soils, inorganic plant constituents, insecticides, water, and separation of nitrogenous bodies (vegetable proteins).)

Nitrogen.

It is recommended—

(1) That the association continue the study of the neutral and alkaline permanganate methods for organic nitrogen availability.

Approved for further study.

(2) That the suggested method for determining nitrogen in nitrates (Bul. 152, p. 28; Cir. 90, p. 2) be further studied.

Approved for further study.

Potash.

It is recommended—

(1) That the proposed modification of the official method (Bul. 152, p. 41) be adopted as official.

Adopted, final action.

(2) That the referee for next year study by pot or plot experiments the relative availability of the potash from different sources, comparing especially the potash of known organic origin with inorganic forms.

Approved.

Phosphoric Acid.

It is recommended—

(1) That further work be done next year on the methods for basic slags with the methods and samples used by the referee this year.

Approved.

Soils.

It is recommended—

(1) That the Rather modification (Bul. 152, p. 52; Cir. 90, p. 3), precipitating the clay in the humus by means of ammonium carbonate, be studied further.

Approved for further study.

(2) That the study of methods of extracting humus and methods for acidity of soils be continued.

Approved.

Inorganic Plant Constituents.

It is recommended—

(1) That the referee for next year be instructed to study further the Schreiber method (Cir. 56) for total sulphur.

Approved.

(2) That further cooperative study of the oxalate method for iron and aluminum be discontinued.

Approved.

(3) That the molybdate method extended to the determination of calcium as oxalate and magnesium as ammonium magnesium phosphate (Bul. 152, p. 62; Cir. 90, p. 5) be further studied.

Approved for further study.

(4) That the molybdate method for the separation of iron and aluminum in an ash solution (Bul. 152, p. 61; Cir. 90, p. 4) be adopted as official.

Adopted, final action.

Insecticides.

It is recommended—

(1) That the chromate method (Method II) for total lead oxid in lead arsenate (Bul. 152, p. 68) be adopted as official.

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[1] Reported Tuesday morning because of the delay of some of the referees' reports.

Adopted, final action.

(2) That further action on recommendations (3), (4), (5), and (6) of the referee for 1911 be deferred pending further study.

Approved. (Available for final action, 1913.)

These recommendations are as follows:

(3) That the method for total sulphur in lime-sulphur solutions (Bul. 107, Rev., p. 34) be changed as follows: Under "2. Determination," line 1, after "Measure," insert "and accurately weigh"; after "sample" strike out "in" and insert a comma and the words "transfer to"; and that the method as thus changed be recommended for final adoption as official in 1912.

(4) That the gravimetric method for sulphur as sulphids and polysulphids in lime-sulphur solutions, as given in the referee's report, be adopted as official.

(5) That the volumetric method for sulphur occurring as thiosulphate in lime-sulphur solutions, as given in the referee's report, be adopted as official.

(6) That the method given (by the referee) for sulphur occurring as sulphates and sulphites in lime-sulphur solutions be changed as follows: Strike out "heat to boiling," etc., to end of paragraph, and substitute "warm on steam bath, precipitate with barium chlorid solution, stirring vigorously for several minutes, let stand in the cold overnight, filter, and obtain weight of barium sulphate. From this weight calculate sulphur," and that the method as changed be adopted as official.

(3) That the provisional methods for the analysis of lead arsenate (Bul. 107, Rev., p. 239) be changed in accordance with recommendation 7 of the referee in 1910 (methods (a) and (b) substituted and (c) and (d) added, as given in Bul. 137, p. 38), and, as changed, be adopted as official.

Adopted, final action.

(4) That the methods (a), (b), (c), (d), (e) for the analysis of lime-sulphur solutions (pp. 36–37) be studied further next year in comparison with the methods as reported by the referee for 1911.

Approved for further study.

(5) That the referee for next year study the method for determining water-soluble arsenic in lead arsenate (p. 37) in comparison with the present provisional method extracting for 10 days.

Approved.

Water.

It is recommended—

(1) That the phenol sulphonic acid method for nitrogen in the form of nitrates (p. 43) be adopted as an official method.

Approved for final action as official in 1913.

(2) That the reduction method for nitrogen in the form of nitrates (p. 43) be adopted as an optional method.

Approved for final action in 1913.

(3) That the method for strontium (p. 44) be further studied.

Approved for further study.

(4) That the colorimetric method for iodin and bromin (p. 44) be further studied.

Approved for further study.

(5) That the methods for analysis of water as published in Circular 52, Bureau of Chemistry, and modified in the Proceedings for 1911 be adopted as official, except the methods for strontium, bromin, and iodin.

Approved for final action as official in 1913.

Separation of Nitrogenous Bodies (Vegetable Proteins).

It is recommended—

(1) That the recommendations of the referee concerning the organization of further work upon methods for the analysis of vegetable proteins be referred to a special committee of three, to be appointed by the Chair, to report upon the practicability of organizing in a special way for the continuous and extended study of the subject.

Approved.

REPORT OF COMMITTEE ON AVAILABILITY OF PHOSPHORIC ACID IN BASIC SLAG.

By C. B. Williams, *Chairman*.

At the last meeting of the association a committee of five, consisting of B. L. Hartwell, of Rhode Island; H. D. Haskins, of Massachusetts; C. G. Hopkins, of Illinois; J. S. Burd, of California; and C. B. Williams, of North Carolina, was appointed to outline and get under way work designed to determine by field, pot, and cylinder experiments the availability of the phosphoric acid in basic slag as compared with that contained in acid phosphate. After much correspondence the committee decided during the spring that as time was short for inaugurating the work it would be well to hold a conference, and early in April a subcommittee, consisting of Messrs. Hartwell, Haskins, and Williams, met in Washington and formulated plans for carrying on the work. As only a little time was available for the committee to get the outline and plans to the workers in most sections before time for spring planting, the chairman of the committee promptly sent out the instructions to a number of station workers in different parts of the country whom he thought to be in a position to take up the work, with the request that if possible the experiments be put out during the spring. The experiments were to be conducted under widely varying conditions of soil and climate. Eight coworkers agreed to conduct the preliminary field work this spring, while three started either cylinder or pot experiments. Many others have indicated since that they will probably be in a position to take up the work next year. The committee feels that the work has been started on a plan and in such a way as to lead to results that will be of far-reaching importance, and regrets exceedingly that because of the early date of this meeting it is unable to give any information as to the results secured from the work of the present year. The work is planned to run for at least five years.

The outline of experiments and plans for their execution are transmitted herewith as a part of the report of the committee. At the beginning of the work it is proposed to secure and store enough basic slag phosphate from four different sources of manufacture to supply the needs of all those cooperating in the experiments with enough of the different slags for the entire period planned to be covered by the investigations.

Outline of Plan for Conducting Field Experiments to Determine Primarily the Availability of Phosphoric Acid in Thomas Slag Phosphate.

Land.—Select land uniform in character and as deficient as possible in phosphoric acid for the experiments. Lay off a rectangular field 220 feet by 158.4 feet as shown in the accompanying illustration. Stake off at least the two plats marked "no fertilizer" (Nos. 1 and 40), to which no applications of lime or fertilizer of any kind are to be made during the progress of the experiment.

Drawing sample.—Take a representative soil sample of the field, making borings to the depth of 8 inches, or to the subsoil, provided the latter is reached at a less depth, excluding surface organic matter. Air-dry the sample, pass through a 3 mm sieve, determine the percentage of coarse material, select a 2-quart sample from the mixed sieved material, dry for 8 hours at 70° C., and preserve for possible future analysis.

Preparation of soil.—Plow to the depth of at least 8 inches, or to the subsoil if that is reached at a less depth, and harrow until the soil is in a fine mechanical condition.

Starting work.—In order to take advantage of the present season and to determine the degree of phosphoric-acid deficiency of the soil, it has seemed advisable not to make the detailed fertilizer applications to each of the plats during the present season, but rather to apply only those materials (lime, nitrogen, and potash) that are common to all plats alike, except Nos. 1 and 40.

Lime.—Add broadcast and harrow in sufficient low-magnesian lime (carbonate or more active forms) to produce a neutral soil during the first season. By those who are in a position to add the lime on the basis of analytical data the following directions may be followed:

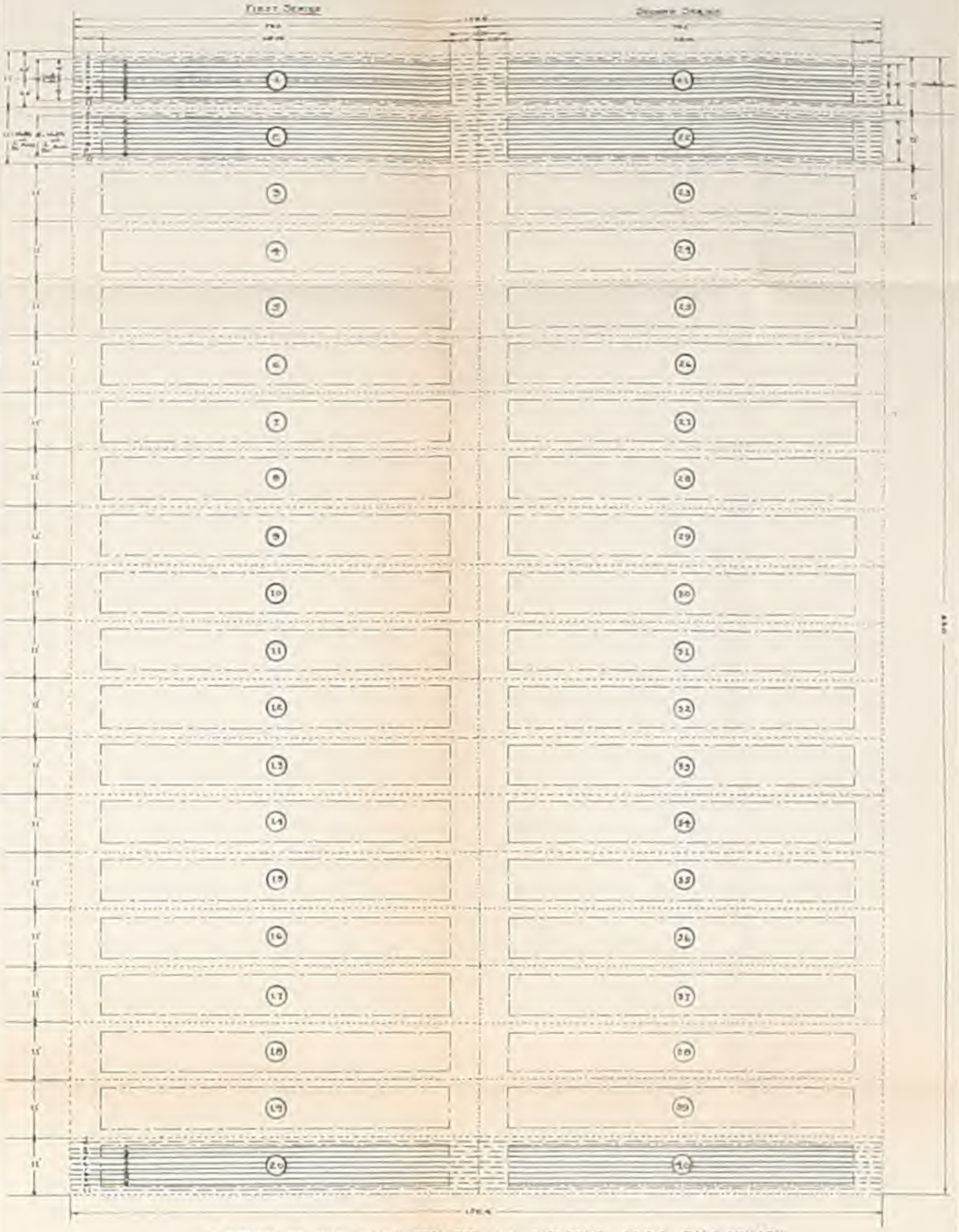
Determine the weight of an acre of soil to the depth used in drawing sample for analysis. Ascertain the lime requirements of the soil in accordance with the Veitch

FIG 1.

-PLAN OF FIELD EXPERIMENT TO STUDY THE AVAILABILITY OF THE PHOSPHORIC-ACID OF BASIC SLAG PHOSPHATE-

SCALE 1-80 APPROX. IS ONE INCH TO A CHAIN

-LEGEND-



A STUDY OF THE AVAILABILITY OF THOMAS SLAG PHOSPHATE

Fertiliser Applications per Plot in Field Experiments

Rates for ¼ Acre Plots

O - No Application of Fertiliser or Lime.
N - 4 pounds Nitrate of Soda + 10 pounds Dried Red Blood
K - 10 pounds Sulphate of Potash, high grade
L - Lime required to neutralise acidity of soil + an additional amount equivalent to 5 pounds of Calcium Oxid
L_1 - Lime required to neutralise acidity of soil + an additional amount equivalent to 5 pounds of Calcium Oxid this year + an additional amount when applications are made next year equivalent to 10 pounds of Calcium Oxid
P - Sub-optimum amount of Phosphoric Acid from the different sources indicated. The normal (P) will have to be fixed by each experimenter for his particular soil

First Series

Plot (1) - C - No Fertiliser
- (2) - NP_1 KL - Phos. Acid from Slag A.
- (3) - NP_1 KL - " " " " B.
- (4) - NP_1 KL - " " " Ground Phos. Rock.
- (5) - NKL
- (6) - NP_1 KL - " " " Acid Phos.
- (7) - NP_1 KL - " " " Slag C.
- (8) - NP_1 KL - " " " Slag D.
- (9) - NP_2 KL - " " " Sol. Phos.
- (10) - $NPKL_4$ - " " " "
- (11) - NPKL - " " " "
- (12) - NP_4 KL - " " " "
- (13) - $N_{\frac{1}{2}}P_{\frac{1}{2}}K_{\frac{1}{2}}L$ - " " " "
- (14) - NPKL - " " " Acid Phos.
- (15) - NPKL - " " " Slag A.
- (16) - NPKL - " " " " B.
- (17) - NPKL - " " " " C.
- (18) - NPKL - " " " " D.
- (19) - NPKL - " " " Ground Phos. Rock.
- (20) - NP_2 KL - " " " Acid Phos.

Second Series

Plot (21) - NPKL - Phos. Acid from Acid Phos.
- (22) - NPKL - " " " Slag A.
- (23) - NPKL - " " " " B.
- (24) - NP_2 KL - " " " Sol. Phos.
- (25) - NPKL - " " " "
- (26) - $NPKL_4$ - " " " "
- (27) - NP_2 KL - " " " Slag C.
- (28) - NP_2 KL - " " " " D.
- (29) - NP_4 KL - " " " Ground Phos. Rock.
- (30) - NKL
- (31) - NP_2 KL - " " " Sol. Phos.
- (32) - NPKL - " " " "
- (33) - $NPKL_4$ - " " " "
- (34) - NP_2 KL - " " " Slag C.
- (35) - NP_2 KL - " " " " D.
- (36) - C - No Fertiliser.

Prepared by the Thomas Slag Analysts Committee of the A. O. A. C.

method (J. Amer. Chem. Soc., 1902, *24* (11): 1120-1128, and U. S. Dept. Agr., Bureau of Chemistry Bul. 73, p. 136), and from these data calculate the amount of calcium oxid required per acre. Before applying this test pass the soil through a 1 millimeter sieve.

The neutralizing power of the lime may be determined by gently heating 1 gram of the material with 50 cc of a standard nitric-acid solution, 1 cc of which is equivalent to 0.5 per cent of calcium oxid, and then titrating the excess of nitric acid against sodium hydroxid solution of an equivalent strength, using Methyl Orange as an indicator, as it is sensitive to both magnesia and lime. In excess of the calculated amount required for neutralizing acidity of the soil, add sufficient lime this year to furnish 250 pounds of calcium oxid per acre, or 5 pounds per plat.

To land adjacent to the experimental plats receiving the same general application indicated for the experimental area this year, including lime, add an additional amount of lime equivalent to 500 pounds of calcium oxid per acre for the purpose of ascertaining if the lime requirements have been fully satisfied in the experimental area. It is also desirable that a liberal application of acid phosphate be made to an adjoining area which has received the general fertilizer treatment given the experimental plats so that a measure of the deficiency of the phosphoric acid in the experimental plats may be secured. The yields from equal areas of the outside plats and the experimental field should be carefully recorded. The results should be tabulated and copies forwarded to the chairman of the Thomas slag phosphate committee as early as possible in the fall.

Nitrogen.—Add broadcast at the rate of 200 pounds of nitrate of soda and 600 pounds of dried red blood (with a content of not less than 13 per cent of nitrogen) per acre.

Potash.—Add broadcast 500 pounds of high-grade sulphate of potash (about 50 per cent potash) per acre.

Crop.—Grow such crops (other than Japanese millet and dwarf Essex rape, which will be used another year after the special fertilizer treatments for the different plats have been made) as will be most effective in depleting the phosphoric acid supply of the soil.

The millet and rape to be used next year, after the special phosphatic materials have been applied, were chosen as crops representing heavy and light feeders of phosphoric acid. Those who intend to grow these crops on separate experimental plats should, of course, lay off the separate areas carefully for the preliminary work this year.

Pot and cylinder experiments.—It is highly desirable that as many as possible conduct pot and cylinder experiments on the same general plan as outlined for the field tests. The applications here of course will have to be much larger per acre, the multiple being governed largely by the size of the pots and cylinders, by the character of the soil, and by the kind of crop.

On the accompanying chart is shown in detail the plan of the experiments which it is proposed to conduct, after it is shown that the soil is sufficiently depleted in phosphoric acid to take up the work. It will probably be desirable to secure at least two years' results after a single application of the special phosphatic materials has been made to the plats.

The report of the committee was received.

REPORTS OF SPECIAL COMMITTEES.

Brief verbal reports were made by the chairmen of the following committees:

Committee on Food Standards.—Mr. Frear, as chairman, called attention to the fact that there were only two (Messrs. Jenkins and Frear) left on the committee, Mr. Wiley having resigned from his official position, thus severing his connection with the association as an active member, and Messrs. Weber and Scovell having died within two weeks preceding the meeting. He said that the committee had met at the American Food, Dairy, and Drug Association meeting and tried to get in touch with the canners' organization which has been making investigations on the fill of canned fruits and vegetables, and that much more work must be done on the composition of raw materials as it affects the liquor in canned foods. He then made the motion that the incoming president be instructed by the association to make appointments to complete the membership of this committee. The motion was carried.

Committee on the Testing of Chemical Reagents.—Mr. Kebler, as chairman, reported that little work had been done in past years on the testing of chemical reagents, the only material made public being a little pamphlet issued by Merck & Co. The number of inefficient chemicals found in the Bureau of Chemistry is very small—not more than 1 per cent—probably because manufacturers realize that the chemicals ordered by the bureau must come up to the specifications, expressed or implied.

A motion made by Mr. Fraps that a referee and an associate referee on testing chemical reagents be appointed was unanimously carried.

The following committees gave no reports and were discharged: Committee on Amendments to the Constitution and By-Laws, Committee on Appropriation, Committee on Presentation of the Question of Unification of Terms to the International Congress of Applied Chemistry, and Committee on Unification of Methods of Analysis of Fats and Oils.

After some discussion, the question of who should do the cooperative work of the association, a motion, by Mr. Davidson, was carried that a committee of three be appointed to draw up resolutions to be sent to the directors of the State experiment stations requesting their cooperation in the work of the association.

Mr. Bigelow suggested that one page of the Proceedings contain the names of former collaborators, with the subjects in which they took an interest. It was believed that such a list would aid the referees.

Mr. Ladd, chairman of the committee appointed to invite the Secretary or the Assistant Secretary of Agriculture and H. W. Wiley to address the association, reported that Assistant Secretary Hays would talk Tuesday at 2 o'clock and Mr. Wiley Wednesday at 11 o'clock.

The association adjourned.

SECOND DAY

TUESDAY—MORNING SESSION.

REPORT ON COLORS.

By W. E. Mathewson, *Associate Referee.*

The work on colors done this year consisted mainly in a trial of our present methods for their separation and identification, a number of colored food samples being examined and reported on by the cooperating chemists. These samples were imitation liqueurs containing sugar, alcohol, and flavoring matter, and were colored as follows, the percentage given below showing the amount of commercial dye in the mixture:

No. 1. 0.01 per cent Amaranth (S. & J. No. 107).
0.01 per cent Ponceau 3R (S. & J. No. 56).
0.01 per cent Naphthol Yellow S (S. & J. No. 4).
0.01 per cent Orange I (S. & J. No. 85). (This dye contained about 5 per cent of the subsidiary color, Orange II, S. & J. No. 86.)
0.01 per cent Erythrosin (S. & J. No. 517).
No. 2. 0.01 per cent Martius Yellow (S. & J. No. 3).
0.01 per cent Naphthol Yellow S (S. & J. No. 4).
0.01 per cent Light Green S F Yellowish (S. & J. No. 435).
No. 3. 0.01 per cent Cochineal (S. & J. No. 706).
0.01 per cent Turmeric (S. & J. No. 707).
No. 4. 0.01 per cent Cyanole Green B (S. & J. No. 491).
0.02 per cent Orange G (S. & J. No. 14).
No. 5. 0.01 per cent Fast Red C (S. & J. No. 103).
0.01 per cent Bismarck Brown (S. & J. No. 197).
No. 6. 0.01 per cent Fluorescein (S. & J. No. 510).
0.01 per cent Rose Bengal (S. & J. No. 523).

It being hardly practicable to send out large amounts of each mixture (more than 4 ounces), somewhat more coloring matter was used than is ordinarily present in such goods. Commercial dyes, previously examined as to their identity, were used in all cases. With the samples was sent a scheme, given below with certain modifications, for the treatment of color mixtures. This is similar in most respects to that tried last year, and intended to group some of the most useful of the solubility relations in a systematic method.

Examination for Coloring Matter.

Before beginning the separation it is usually best to obtain the coloring matter in solution. If this is done with dilute alcohol or ammoniacal alcohol, the alcohol must be removed on the steam bath before beginning the extraction with immiscible solvents. From many solid food products, however, the common dyes may be taken up by shaking the well-divided material with amyl alcohol containing a little concentrated hydrochloric acid.

Separation.

Make a small portion of the mixture alkaline with sodium hydroxid and shake with ether; separate ether and make acid with acetic acid. If the ether solution is colored, or yields a coloring matter to the acetic acid, indicating the presence of a basic color,

treat a larger portion of the sample in the same manner, extracting several times with ether to remove all basic color. Shake out the combined ether extracts with successive small portions of water, and finally with dilute acetic acid. If more than one basic color is present, they will usually be more or less completely separated by this treatment, this being indicated by the difference in color, fluorescence, etc., of the succes sive portions. Those fractions containing the chief amount of each color are combined and refractionated again if necessary in the same way until some of each color has been obtained in pure condition. The dyes are then identified by the usual methods. The ether solution of the color bases may also be shaken out directly with very dilute hydrochloric acid, the acid extract shaken with amyl alcohol, and the latter solution washed successively with fourth-normal, normal, and four-normal hydrochloric acid.

Treat the original mixture, free from basic colors, with half its volume concentrated hydrochloric acid and shake out with not too large portions of amyl alcohol until no more color seems to be extracted; two or three shake outs are usually enough. First wash the combined amyl alcohol extracts with a little hydrochloric acid (one volume strong acid to two of water) to remove sugar, etc., then shake out with successive portions of water of perhaps one-half the volume of amyl alcohol until the last few portions are perfectly neutral. Eight or ten fractions will usually be obtained. Then dilute the amyl alcohol with one or two volumes of gasoline or petroleum ether and shake out first once or twice with water, finally with a very dilute caustic soda solution.

In this treatment most of the acid dyes are taken up by the amyl alcohol and come out on washing with water, in general the higher sulphonated ones first while the wash waters still contain much hydrochloric acid, the lower sulphonated ones later, and finally the unsulphonated acid colors as Erythrosin, Martius Yellow, etc. If a sufficient separation has not already been effected, the fractions containing the chief amount of each color are united, acidified, taken up with amyl alcohol, and again shaken out with water (or with hydrochloric acid of suitable strength). With these fractions coming out last, ether or petroleum ether is used instead of amyl alcohol. Occasionally in washing the amyl alcohol solution the liquids do not separate readily. In this case the mixtures may be poured out in a beaker and warmed, or hot water may be used. From hot mixtures, however, most dyes are extracted at higher acidity, and it is usually better to use a centrifuge. It must be remembered that a variation in color in the different fractions does not always indicate the presence of more than one dye, as it may merely be due to the difference in acidity. This, of course, can be decided by treating a few cubic centimeters with sodium acid carbonate. As it is advantageous to keep the color solutions as concentrated as possible, unnecessarily large amounts of the solvents should not be used. The total original amyl alcohol extract need not exceed 50 to 75 cc.

Of the permitted dyes, Indigo Carmine is most quickly removed from the amyl alcohol on washing, the order being Indigo Carmine, Amaranth, Ponceau 3R, Naphthol Yellow S, Orange I, Erythrosin.

If all three colors, Ponceau 3R, Naphthol Yellow S, and Orange I, are suspected of being present, they are most readily separated by treating the combined fractions containing them with one-fourth volume of sodium chlorid solution (250 grams per liter) and shaking out with one or two portions of amyl alcohol, which will take up the Orange. The amyl alcohol is washed with 5 per cent salt solution to remove any Yellow or Ponceau, then two or three times with normal (5 per cent) sodium carbonate solution. This will take out all Orange I, forming a deep crimson solution, but will leave the bulk of any Orange II, Crocein Orange, etc., present, in the amyl alcohol. The original combined portions from which the Orange has been removed with amyl alcohol are now treated with one-tenth to one-fifth volume concentrated hydrochloric acid and the Yellow taken out by two or three shakings with amyl acetate. The Yellow is removed from the amyl acetate by washing with water. Ethyl acetate may be used instead of amyl acetate, in which case less hydrochloric

(perhaps about one-fortieth volume) should be added to the salt mixture. It must be remembered that Naphthol Yellow S in acid solutions is pale yellow or colorless. From the acid salt solution the Ponceau may be taken up with a little amyl alcohol, this washed once with water, then diluted with gasoline, and the color taken out with water. A small portion of this solution treated with a few drops of barium acetate solution should give a deep purplish red flocculent precipitate, the supernatant liquid being colorless. Ponceau 2R reacts similarly, the precipitate being Carmine Red, however.

Amaranth is readily separated and distinguished from the Ponceau and Fast Reds by its different distribution ratio between amyl alcohol and dilute hydrochloric acid. A dilute solution of Ponceau 3R in fourth-normal hydrochloric acid, when shaken with an equal volume of amyl alcohol, yields practically all of the color to the latter. Amaranth under similar conditions remains for the greater part in the lower layer. Indigo Carmine is not readily taken up from slightly acid solutions by dichlorhydrin (difference from common blue and green triphenylmethane and azin dyes).

The original mixture from which basic dyes have been removed with ether and acid dyes with amyl alcohol and which perhaps appears perfectly colorless may still contain Light Green S F, Yellowish, and some other dyes. Make slightly alkaline with sodium carbonate or ammonia, then acidify slightly with acetic acid and shake out once or twice with amyl alcohol to remove any Guinea Green, Methylene Blue, etc., present. Then extract with one or two small portions of dichlorhydrin that will take out Light Green S F and similar strongly sulphonated triphenylmethane greens. The dichlorhydrin may be diluted with double its volume of benzol and the dye washed out with water. Some of the rather uncommon mono- and di-sulphonated triphenylmethane and azin dyes, being more readily extracted from slightly acid than strongly acid solutions, will perhaps appear both in the first and the final washings of the amyl alcohol. These colors may be separated readily by shaking the fractions containing them with dichlorhydrin. In most cases they are extracted by amyl alcohol from neutral solutions containing 5 per cent or more sodium chlorid

Natural coloring matters are often mixtures of several chemical individuals of similar color but different solubilities. Further, some of these substances may have no pronounced acidic or basic character, hence their distribution ratio between amyl alcohol and water is not affected greatly by addition of hydrochloric acid. In the routine treatment as described, Archil (unsulphonated), Turmeric, and Saffron will be taken up by the amyl alcohol and removed from this when, after dilution with gasoline, it is shaken with caustic soda. The alkaline solution may be drawn off, acidified, and shaken out with amyl alcohol.

After separating the solution and driving off the solvent on the steam bath, the residue consisting of the coloring matter fairly free from impurities may be tested by the usual methods. The coloring matters of cochineal and of Persian berries are gradually removed (Persian berries incompletely) by the washing with water, and are conveniently identified in the solutions so obtained, especially in the fraction obtained after dilution with gasoline. A number of natural coloring matters are, like Naphthol Yellow S, rendered much paler by acids and may be overlooked in the washings. In acid solution most natural coloring matters are deepened in tint on addition of stannous chlorid, while most of the common coal-tar dyes are decolorized, and it is usually advisable to test a few drops of the strongly colored fractions with this reagent.

In the color fractionation as described, it must be remembered that any given dye will, in general, appear in several washings. Where the maximum amount comes out, however, may be shown by the following table, the dyes being designated by the numbers given in the Green-Schultz-Julius tables, second edition, 1904.

Outline.

BASIC DYES. EXTRACTED BY ETHER FROM STRONGLY ALKALINE SOLUTIONS.

(Extracted only in small amount, perhaps with decomposition: 650.)

A. Readily removed from ether on washing with water: 448, 584.

B. More or less slowly removed by water, quickly by acetic acid: 425, 427, 428, 451, 452, 504, 655.

C. Not removed by water, rather readily removed by acetic acid: 17, 18, 197, 201.

D. Not removed by acetic acid, fairly readily removed by hydrochloric acid (oil-soluble colors): 7, 16.

E. Not removed by hydrochloric acid (oil-soluble colors): 11, 49, 60.

ACID DYES. NOT EXTRACTED BY ETHER.

A. *Extracted by amyl alcohol from the strongly acidified solution.*

1. Removed in first washings of amyl alcohol extract, acidity high: 8, 9, 89, 108, 692.
2. Removed at lower acidity, but usually above fourth-normal: 94, 106, 107, 602.
3. Removed at rather low acidity: 14, 53, 188, 480.
4. Removed at very low acidity, but before washings are neutral. Like preceding acid colors, not extracted by amyl alcohol from 5 per cent sodium chlorid solution.
    a. Removed from strongly acidified solution by amyl acetate: 4.
    b. Not readily removed by amyl acetate: 55, 56, 62, 64, 65, 84, 103, 105, 139.
5. Removed by water from the practically neutral solvent, most readily after addition of petroleum ether.
    a. Not completely extracted by amyl alcohol from 5 per cent salt solution: 146, 169, 667.
    b. Almost completely extracted:
        x. Extracted by 5 per cent sodium carbonate solution from amyl alcohol: 85.
        y. Not readily extracted: 13, 86, 95, 97, 101, 137, 329. (464, 468, 433.)
6. Removed by dilute sodium hydroxid solution from the amyl alcohol-petroleum ether mixture. Readily extracted by ether from acid solutions: 2, 3, 269, 510, 512, 516, 517, 518, 520, 523.

B. *Not extracted from the strongly acid solution by amyl alcohol.*

1. (Decomposed: 398.)
2. (Dye separates as a precipitate, but is extracted by dichlorhydrin: 240, 602.)
3. After adding ammonia until nearly neutral:
    a. Readily extracted by amyl alcohol: 433, 464, 468, (650).
    b. Not readily extracted by amyl alcohol:
        x. Extracted by dichlorhydrin: 434, 435, 440, 491.
        y. Not readily extracted by dichlorhydrin: 462.

Cooperative Work on Colors, 1912.

The reports of the analysts taking part in the work this year are given in the following table:

Cooperative work on colors, 1912.

Analyst.	Coloring matters reported.					
	Sample 1.	Sample 2.	Sample 3.	Sample 4.	Sample 5.	Sample 6.
E. H. Grant, New Orleans, La.	Amaranth. Orange I. Erythrosin (?)	Naphthol Yellow S. Light Green S F Yellowish.	Cochineal. Turmeric.	Crocein Orange G. Tetracyanole S F.	Fast Red C. Unidentified Orange color.	Fluorescein. Rose Bengal.
H. M. Loomis, Seattle, Wash.	Amaranth. Ponceau 3 R. Naphthol Yellow S. Orange I. Orange II. Erythrosin.	Naphthol Yellow S. Light Green S F Yellowish. Martius Yellow.	Cochineal. Turmeric.	Orange G. Cyanole Green 6 G.	Fast Red C. Bismarck Brown.	Fluorescein. Rose Bengal.
D. L. Weatherhead, Bozeman, Mont.	Amaranth. Ponceau 3 R. Naphthol Yellow S. Orange I. Orange II. Erythrosin.	Naphthol Yellow S. Light Green S F Yellowish. Martius Yellow.	Cochineal. Turmeric.	Orange G. Cyanole Green B.	Fast Red C. Bismarck Brown.	Fluorescein. Phloxin.
C. S. Brinton, Philadelphia, Pa.	Amaranth. Ponceau 3 R. Orange I. Erythrosin.	Light Green S F Yellowish. Naphthol Yellow S. Martius Yellow.	Cochineal. Turmeric.	Tropaeolin O. Tetracyanole S F.	Bismarck Brown. Amaranth.	Fluorescein. Rose Bengal.
W. C. Burnet and L. Patton, Savannah, Ga.	Amaranth. Orange I. Erythrosin.	Light Green S F Yellowish. Naphthol Yellow S.	Alizarin Red S. Turmeric.	Orange G. Light Green S F Yellowish.	Bismarck Brown. Amaranth.	Phloxin.

It was necessary in nearly all cases to request that a supplementary report be made on Sample 1. This was perhaps largely due to the fact none of the analysts suspected the presence together of so many colors of not widely different shades and because in the fractionation with amyl alcohol by the methods sent out the Naphthol Yellow S comes out with the Ponceau, and this mixture is very similar in shade to the Orange I, the bulk of which comes out later.

H. M. Loomis, whose first report was nearly correct, made a preliminary separation of the colors in all the mixtures except No. 6 by the method sent, the identification of the separated dyes by the scheme and data given in Circular 63. In Mixture 1, however, he used ethyl acetate, instead of amyl acetate, to separate the Yellow, obtaining satisfactory results. Amyl acetate was recommended in the scheme, as it was supposed that the ethyl ester was too rapidly saponified in acid mixtures to be suitable. Shaken with five-normal hydrochloric acid, ethyl acetate seems to be much more rapidly attacked, perhaps because of its higher solubility. The dyes in Sample 6 were separated by making the aqueous solution alkaline with ammonia and shaking with amyl alcohol, the pink color being completely removed after four or five shakings. After separating the acid colors with amyl alcohol, he found it advisable in most cases to wash the combined amyl alcohol extracts with hydrochloric acid (one volume acid to two of water) to remove sugars, etc., before beginning the shaking out with water.

Bearing on the separation of the permitted dyes, C. S. Brinton said he had tried the method of separation given on page 3 (of the scheme sent) as outlined for this year involving the use of sodium carbonate and amyl acetate and, as far as he had been able to go, he believed that this method would satisfactorily separate some of the permitted

colors, although he had not been able thus far to satisfactorily get out Ponceau 3 R, owing probably to lack of time and inexperience with the method, rather than to the fault of the method itself.

E. H. Grant stated that the unidentified Orange dye found in Mixture 5 was extracted by ether from alkaline solution. Possibly the difficulty in identification was due to the fact that, Bismarck Brown being a mixture of several substances, the dye obtained in the separation did not correspond exactly in reactions to known samples at hand. He separated the dyes in No. 6 by making alkaline with sodium carbonate and extracting repeatedly with ethyl acetate. The combined ethyl-acetate solutions were washed first with sodium carbonate, which was added to the original alkaline solution, and then with water, which extracted the pink color. The fractionation was continued in the same manner and the last traces of red dye finally removed from the uranin solution with wool.

D. L. Weatherhead separated the dyes in Mixture 6 by merely continuing the washing of the amyl-alcohol extract with hot water, the Rose Bengal being first removed, leaving the Fluorescein in solution.

Examination of the results given indicates that most of the errors were due not to imperfect separation, but to confusion of nearly similar dyes in making the identifications. The separation of the Eosin dyes suggested in the scheme, by shaking the solution of their color acids in ether with a solution of secondary sodium phosphate, containing some sodium acid phosphate, was not tried, and probably some of the other methods used are better.

In connection with the adoption of a method, it may be said that the analyst ordinarily knows little regarding the nature of the coloring matters present in a sample, and the treatment must be such that in case a dye mixture was used none of the constituents will escape detection. The plan to be followed must take into consideration the following points: It must require comparatively little time and manipulation when only permitted dyes are present, and separate these satisfactorily. By far the larger part of the samples met in routine work contain only permitted colors, perhaps most frequently in admixture with each other. The chief object in the color examination being, in the majority of cases, to determine whether or not nonpermitted dyes are present, it must so far as possible give separations of these from the permitted dyes, and from each other. It should allow not merely a detection of the colors present, but a separation, so that each dye may be fixed on fiber and retained for subsequent reference.

It must avoid the use of reagents likely to modify or destroy the coloring matters, and, finally, must enable the analyst to judge something of their chemical nature. In the opinion of the writer, methods employing immiscible solvents meet these requirements much better than those based on the use of precipitants or textile fibers. The former are likely to give amorphous precipitates, occluding considerable amounts of soluble dyes and being purified with difficulty. Dyeing and stripping is usually slow, accompanied by the loss of much dye, and practically worthless, except for the simplest mixtures. With the methods described, when only one coloring matter is present, much cleaner and brighter dyeings can usually be obtained from the fractional washings than can be secured by double dyeing. Emulsification with the amyl alcohol is prevented in most cases by the acid present.

Benzidin and lower sulphonated triphenylmethane dyes might be more easily removed otherwise, but are seldom encountered. B. C. Hesse (Bureau of Chemistry Bul. 147, pp. 19–20) gives data regarding 170 samples of food colors on the market in 1907. Thirty-two were of dyes now permitted. Of the other 138, 65 were monazo colors, 16 disazo, 2 nitroso, 3 diphenylmethane, 26 triphenylmethane, 20 xanthene, 2 azin, 3 thiazin, and 1 quinolin. These samples represented the stock offered by a number of manufacturers, but the numbers do not show the relative amounts sold. They indicate, however, that the low-priced azo dyes are much more widely used than

those of any other class. Data taken by permission of the chief from the records of the New York laboratory show that during a certain period in which nonpermitted dyes were reported in 80 instances they were classified as follows:

Kind of dye.	Acid.	Basic.	Total.	Kind of dye.	Acid.	Basic.	Total.
Nitro	1	0	1	Xanthene	3	4	7
Monazo	49	1	50	Azin	2	0	2
Disazo	1	1	2	Quinolin	4	0	4
Diphenylmethane	0	1	1				
Triphenylmethane	8	5	13	Total	68	12	80

Regarding identification of the coloring matters, Circular 63 and the standard works of reference give data whose value is generally recognized and requires no comment. It is believed by the writer that with the knowledge that is at hand after the separation has been effected the color reactions given by the common reagents on the dyed fiber are sufficient in nearly all cases with the 60 or 70 colors in common use. Some experience on the part of the analyst is required, and of course supplementary tests should always be used for confirmation. With the monazo dyes, an examination of the reduction products nearly always gives positive results with amounts of color equal to 1 mg or more, and would seem to merit more general use. Oxidation with bromin or chlorin water (removing excess with a few drops of hydrazin sulphate solution) and coupling the diazo compound formed with a naphthol also gives a very sensitive and characteristic reaction for many of the azo dyes.

Although commercial dyes containing subsidiary coloring matters in greater or less amount are used in food manufacture, it is evident that the latter will usually be separated in the analysis, and it is therefore important in the identification to know the reactions of the chemical individuals. Further work in this direction seems desirable.

It is recommended that the method described, for the qualitative separation of coloring matters, be adopted provisionally by the association, and that work bearing on the separation and identification of these substances be continued.

REPORT ON SACCHARINE PRODUCTS.

Mr. Chittick, the referee on saccharine products, made no formal report but recommended that the suggestions of the referee for 1911 as to further study be adopted and that the method for the determination of solids in molasses and other sugar products, by means of the refractometer, using Geerlig's table of equivalents and temperature corrections, be adopted as provisional, but that the results be expressed as percentages calculated from the refractometer readings.

NOTE ON THE ANALYSIS AND VALUATION OF MAPLE SUGAR.

By A. H. Bryan.

When examining samples of maple sugar for purity, it has been found best to dissolve about 100 grams of the sugar in about 400 cc of water with heat and then to concentrate about one-half, removing any scum that may rise but being careful not to remove any crystals of sugar. The solution while hot is passed through a coarse filter paper to remove any sediment and concentrated to a solid content of at least 65 per cent. This is ascertained in the Sugar Laboratory of the Bureau of Chemistry by noting the temperature at which the solution boils. A 65 per cent solid content

maple sirup boils at 103.5° to 104.5° C. The hot solution is allowed to cool and settle. The clear liquid is then subjected to the regular analysis for maple sirups, and the values attached to maple sirups are then applicable to maple sugar.

C. H. Jones,[1] of the Vermont Experiment Station, in 1904 called attention to the fact, which is of extreme importance, that the sugar should be analyzed as a sirup and not as sugar, for the reason that in the manufacture of maple sugar less care is generally used to filter or strain out the precipitated "sugar sand" (malate of lime) which finally goes into the crystallized product but would be removed in the manufacture of sirup. The work of the Bureau of Chemistry along this line bears out the original work of Mr. Jones, and it seems proper that all maple sugars should be analyzed in the sirup condition and the results calculated to the dry basis. The percentage of moisture in the sugar can be determined directly by the usual methods.

REPORT ON FRUIT PRODUCTS.

By H. C. Gore, *Associate Referee.*

Studies on the Determination of Citric and Malic Acids.

Remarkable progress has been made during the last two years on methods for the estimation of the organic acids of fruit, cane, and maple products.

Yoder [2] has found that succinic, aconitic, and lactic acids are readily removed from their water solutions by perforation with ether, while malic, citric, and tartaric acids are so extracted very slowly. Precipitation tests were made with the calcium and certain other salts of all these acids; aconitic acid was found to be the predominating organic acid in the juice of sugar cane.

Bacon and Dunbar [3] have studied the organic acids of fresh and spoiled tomatoes. Citric acid was found to be the only organic acid present in appreciable amounts in sound tomatoes, while lactic acid was found in many of the large number of spoiled tomato pulps and ketchups examined. A simple method proposed for the determination of citric acid in tomato products consisted in precipitating citric acid in 50 to 60 per cent alcohol solution by the addition of barium acetate, collecting, washing, drying, and weighing the barium citrate. Lactic acid was determined by removing it first by perforation under proper conditions with ether, then by oxidation with solution of alkaline permanganate. Oxidation to oxalic acid and carbon dioxid occurs. The solution is acidified with sulphuric acid, when the oxalic acid is oxidized. Excess of permanganate is measured by titration with oxalic acid.

Pratt [4] has developed a gravimetric method for the determination of citric acid probably applicable to a wide range of fruit products. This method has been sent out for trial this year. Briefly, it consists in precipitating the citric acid in 50 per cent alcohol solution by addition of solution of barium acetate, collecting the precipitate, dissolving in phosphoric acid, and adding a portion estimated to contain from 50 to 150 mg of citric acid to a large volume of hot water in a distilling flask. Distillation is then carried on while the citric acid is oxidized by slow addition of dilute solution of permanganate. Acetone is formed and collects in the distillate, in which it is precipitated by treatment with Denigé's reagent. The mercury compound which separates is filtered off, washed, dried, and weighed, and the weight multiplied by the factor 0.22.

Yoder [5] called attention to the statement by Walden [6] that uranium salts possess the property of entering into combination with malic acid so as to increase its rotatory

[1] 17th Annual Report, Vt. Expt. Station, 1904, p. 453. 18th Annual Report, Vt. Expt. Station, 1905, p. 327.

[2] J. Ind. Eng. Chem., 1911, *3:* 640.

[3] U. S. Dept. Agri., Bureau of Chemistry Cir. 78.

[4] U. S. Dept. Agri., Bureau of Chemistry Cir. 88.

[5] J. Ind. Eng. Chem., 1911, *3:* 563; Zts. Nahr. Genussm., 1911, *22:* 329.

[6] Ber. d. Chem. Ges., 1897, *30:* 2889.

power many fold. Yoder found a 229-fold increase in the specific rotatory power of malic acid, from $(\alpha)\frac{20}{D}=-2.25$ to $(\alpha)\frac{20}{D}=-515$, and developed the necessary fundamental facts upon which analytical procedures for the determination of malic acid may be based. The following topics were discussed: Influence of concentration of the malic acid, of the kind and quantity of uranyl compounds used, of acids and alkalies, of sugars, of time of standing after addition of uranium salt, of heating in dissolving the uranium salt, and of kind of light used in polarizing, and some notes on the polarization of tartaric acid in the presence of uranium salts. A general discussion is then given, followed by details of methods proposed for the estimation of malic acid in cane and maple products and analytical data on typical samples of cane and maple sirups.

Dunbar and Bacon [1] subsequently published a method for the estimation of malic acid in fruit products based on the effect of uranium salts on the polarization of malic acid. This method has been sent out for trial during the past season.

Determination of Citric Acid.

The data secured by the collaborators in the study of the determination of citric acid in orange juice by the Pratt method are given below:

Determination of citric acid in orange juice by the Pratt method.

Collaborator.	Citric acid.				
	Determination 1.	Determination 2.	Determination 3.	Determination 4.	Determination 5.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
J. M. Johnson, Washington, D. C.	(a) 0.62 (b) .79	0.89	(a) 0.86 (b) .79	0.76	0.84
Geo. E. Colby, Berkeley, Cal.	.93				
C. H. McCharles, Berkeley, Cal.	.91				
P. A. Yoder, Washington, D. C.	(a) .824 (b) .836	.787	.840		
P. B. Dunbar, Washington, D. C.	.72	(a) .73 (b) .71	(a) .63 (b) .72	(a) .69 (b) .76	
F. F. Fitzgerald, Washington, D. C.	.80	.80	.76	.79	.85
H. C. Gore, Washington, D. C.	.81	(a) .80 (b) .73	(a) .87 (b) .86	(a) .80 (b) .84	(a) .79 (b) .89
A. R. Todd, Lansing, Mich.	.834				

Note.—The sample of orange juice contained 0.83 per cent of free acid as citric, phenolphthalein as indicator. The total alkalinity of the ash, expressed as potassium carbonate, was 0.37 per cent.

COMMENTS OF COLLABORATORS.

J. M. Johnson: The results are not very close, and I do not believe the method can be used for quantitative work.

P. A. Yoder: Nos. 1 a and 1 b were aliquots of the same solution of barium citrate. In No. 1 b less potassium permanganate was used than in No. 1 a, none having been added after a pink color persisted for from 20 to 40 seconds, while in No. 1 a a slow stream of potassium permanganate solution was continued to near the end of the distilling process, so as to maintain a very light pink color in the solution. It is noted that in the former instance slightly higher results were obtained. In No. 2, where lower results were obtained than in the others, the time elapsing between the addition of barium acetate and the filtration was only about 15 minutes, while in Nos. 1 and 3 it stood overnight at this stage.

Recalling that in some of my former work on organic acids [2] such preparations of aconitic acids as I then had on hand gave a reaction with Denigé's reagent similar to that with citric acid, the question arises whether possibly this acid and also tricarballylic acid, which are both closely related to citric acid in constitution, might not also yield a product by distillation into Denigé's reagent the same as citric acid. In an

[1] U. S. Dept. of Agr., Bureau of Chemistry Cir. 76. [2] J. Ind. Eng. Chem., 1911, *3*: 644.

attempt to answer the question I made a distillation with a commercial preparation of aconitic acid, the same as in the determination for citric acid, and found it to yield a precipitate equivalent to 8.41 and 8.24 per cent of citric acid respectively in the duplicates or computed as aconitic acid to 7.61 and 7.47 per cent of the weight of the dry acid taken. The titration of the solution agreed with the weight of the dry acid. It still remains unanswered whether this is really a contamination of so much citric acid in the sample, or whether so small a portion of the aconitic acid reacts like citric acid. From the close agreement of the duplicate and the low percentage it seems most probable that a contamination of citric acid is actually present.

Relative to the precipitation with alcohol prior to the addition of barium acetate, the question suggests itself whether some citric acid may not be precipitated here, owing to the presence of alkali earth metals, namely, lime, in the juice. This is to be feared the more if the juice is high in lime content and low in total acids. Should such loss of citric acid occur, then it could be obviated, I anticipate, by the addition of a small amount of hydrochloric acid, which could be again neutralized after filtration, before precipitation with barium acetate.

P. B. Dunbar: In the first and second determinations the details of the method were followed exactly as described. In the third determination, citric acid was precipitated in 74 per cent instead of 50 per cent alcohol, while in the fourth determination 60 per cent alcohol was used.

F. F. Fitzgerald: Method followed exactly except that in some cases the filtration to remove pectin bodies was done using a Büchner funnel. From my experience with this method, the duplicates in this case check more closely than usual.

H. C. Gore: Exact procedure followed except that filtrations were conducted in a Büchner funnel, using suction. In collecting the precipitate of barium citrate, it was found advantageous to fit the Büchner funnel with a filter paper about 1 cm wider than the interior diameter of the funnel, the edges of the paper projecting upward, thus forming a shallow cell in which the precipitate was collected.

DISCUSSION.

F. F. Fitzgerald and J. M. Johnson stated that the method became unreliable when large amounts of tartaric acid were present, as then oxidation products which also give a precipitate with Denegi's reagent were formed from tartaric acid. These collaborators submit the following data in support of this statement:

Collaborator.	Tartaric acid oxidized as in Pratt method.	Weight of precipitate formed with Denigé's reagent.
	Grams.	*Grams.*
F. F. Fitzgerald	0.200	0.01
	.200	.12
	.400	.33
J. M. Johnson	.100	0
	.200	0
	.500	Turbidity.
	1.000	1.41

This unreliability would seem to limit the application of the method to fruit products free from appreciable amounts of tartaric acid, unless tartaric acid is removed before oxidizing.

The results of Yoder on aconitic acid show that although this acid, even though closely related to citric acid, probably does not seriously interfere. The question is still open.

A serious defect in the method is the difficulty encountered in getting good duplicates. The difficulty is met with even when two equal portions of the same solution of barium citrate are run side by side, indicating that minor changes in method of oxidation are probably responsible. It is suggested that the conditions of oxidation in particular receive attention in the further study of the method.

Determination of Malic and Tartaric Acids.

DUNBAR AND BACON METHOD FOR DETERMINATION OF MALIC ACID.

An outline of the procedure described by Dunbar and Bacon is as follows:

Take 75 cc of sample, neutralize, and dilute to 100 cc. To an aliquot of 25 cc add about 2.5 grams of uranium acetate, allow to stand with occasional agitation for about 2 hours, filter, and polarize. Clarify the remainder of the solution by adding dry lead acetate, avoiding an excess, filter, and remove lead with dry sodium sulphate. Polarize the filtrate. The difference between the polarizations made in 200 mm tubes, or calculated to this tube length, times 0.036, gives the malic acid present in terms of grams per 100 cc.

Sugars were found to suffer an apparent (see p. 69) decided reduction in rotation on the addition of the uranium salt, and in the presence of large amounts of sugar it is directed to remove malic acid as completely as possible by cooling in ice water before filtering after the addition of lead acetate. After the removal of excess of lead, the solution is treated with uranium acetate. (If the polarization of the original solution is positive, this procedure is not followed.) If the reading is less than the reading made after the removal of the greater part of the malic acid, it is used in the calculation; if greater, then the original reading, made after treatment with lead acetate, is employed (see p. 69).

The sample of cider sent to the collaborators for the estimation of malic acid contained 0.50 per cent of free acid as malic, phenolphthalein as indicator. The total alkalinity of the ash, expressed as potassium carbonate, was 0.26 per cent.

Determination of malic acid by Dunbar and Bacon method.

Collaborator.	Malic acid.		
	Determination 1.	Determination 2.	Determination 3.
	Per cent.	*Per cent.*	*Per cent.*
J. M. Johnson, Washington, D. C.	0.48	0.50	0.47
Geo. E. Colby, Berkeley, Cal.	.497	.....	.....
C. H. McCharles, Berkeley, Cal.	.497	.....	.....
P. A. Yoder, Washington, D. C.	.468	[1].49	.....
F. F. Fitzgerald, Washington, D. C.	[1].48	[1].48	[1].49
P. B. Dunbar, Washington, D. C.	[2].48	[2].51	.....
H. C. Gore, Washington, D. C.	.504	.....	.....
A. R. Todd, Lansing, Mich.	.531	.....	.....

[1] By Pratt modification. [2] Not neutralized before adding uranium salt.

COMMENTS OF COLLABORATORS.

J. M. Johnson: I think the method works very well; the use of bromin in destroying color is, however, not advisable, as it may cause low results. More work should be done on the effect of bromin.

P. A. Yoder: Duplicate determinations were made both by the straight Dunbar and Bacon procedure and by the Pratt modification of this procedure. In the first determinations agreement between duplicates was not good, and only in the third sets, Nos. IX, X, XI, and XII, were fairly satisfactory duplicates obtained. On the chance, however, that the other results may be of interest to you, I submit them also in the accompanying table. The results in italics are the ones to be used, according to the directions by the authors. The marks "d," "dd," and "ddd" after polarization numbers indicate relative intensities in color of solutions which offered difficulties in polarizing. From these results, taking the average of the last sets in each case, I will report for the straight Dunbar and Bacon procedure 0.492 gram in 100 cc, or 0.468 per cent by weight. By the Pratt modification, when the solution was not neutralized before treatment with uranium acetate, I found 0.533 gram in 100 cc or 0.507 per cent by weight, and when it was neutralized 0. 515 gram per 100 cc or 0.490 per cent by weight. (The circular describing the Pratt modification of the Dunbar and Bacon procedure is not explicit as to whether or not this neutralization, included in the Dunbar and Bacon procedure, should also be made here. On the basis of the results obtained in the previous work I anticipated a difference in the results, which anticipation was verified.) The specific gravity of the sample was found to be 1.051.

Summary of results on malic acid in cider (Yoder).

BY STRAIGHT DUNBAR AND BACON PROCEDURE, USING 75 CC.

Notebook No.	Reading 1: With uranium acetate.		Reading 2.		Reading 3: With uranium acetate after clarification with lead acetate.	Grams malic acid per 100 cc.				
						Using Reading 2.		Using Reading 3.		
	Not previously neutralized.	Neutralized with sodium hydroxid.	Without clarification, except with aluminum hydroxid.	With lead acetate and cooling clarification.		From columns (2) and (5).	From columns (3) and (5).	From columns (2) and (6).	From columns (3) and (6).	From columns. (3) and (4).
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
I	.....	39.21	27.54dd	27.74	28.33	.....	*0.550*	.....	0.522	0.560
II	.....	39.72	27.34dd	27.55	27.91	.....	*.594*	.....	.560	.588
V	.....	38.29dd	26.46ddd	28.11	29.01	.....	*.489*	.....	.445	.568
VI	.....	37.89dd	27.65dd	27.52	27.65	.....	*.498*	.....	.492	.492
IX	.....	38.18dd	27.72	27.82	29.00	.....	*.497*	.....	.441	.502
X	.....	38.07	27.44	27.93	29.28	.....	*.487*	.....	.421	.510

BY THE PRATT MODIFICATION OF THE DUNBAR AND BACON PROCEDURE, USING 100 CC.

III	47.82d	47.66d	.....	34.40	37.17	*0.482*	*0.477*	0.383	0.378	.....
IV	50.06d	49.54	.....	34.62	36.78	*.556*	*.537*	.478	.460	.....
VII	49.82ddd	49.14d	.....	34.93	34.89	.536	.512	*.537*	*.513*	.....
VIII	48.74dd	47.90	.....	34.36	34.62	*.518*	*.487*	.508	.478	.....
XI	49.94d	49.23d	.....	34.94	37.21	*.540*	*.514*	.458	.433	.....
XII	48.55	48.28	.....	33.94	36.44	*.526*	*.516*	.436	.426	.....

Supplemental to these determinations of malic acid in the cider, I made some tests with a preparation of malic acid, presumably pure, to redetermine the activity of the uranium compound. I used a tenth-normal solution, the strength of which was established by titration with a standard alkali, and I added in each case uranium acetate equivalent to 1.5 atoms of uranium to 1 molecule of malic acid. Duplicates not neutralized gave a levo reading of 19.11 and 19.11, respectively, with light from an incandescent electric lamp, equivalent to 28.51 for a 1 per cent solution, and of 18.96 and 18.96 with yellow sodium light, equivalent to 28.29 for a 1 per cent solution.

Comparison shows this sodium light value to be a little lower than that I obtained in some earlier work, namely, 28.95.[1] Whether this is due to inaccuracies in the determinations in either the former case or this case, or is due to some acid contamination in the preparation of the malic acid now available, remains undetermined. The method of making up the solutions and ascertaining their strengths was the same in the two instances. It should be noted in this connection that in the former work lower results were also obtained when a preparation from another source was used [2] than in the final determination with what was presumably the purest preparation.[3] It was also noted that in dissolving a sample of malic acid now on hand in a small quantity of water a slight cloudiness remained which cleared up on addition of more water, an evidence of an impurity.

The white-light values obtained now (28.51) and in the previous work (29.72) are not strictly comparable since different sources of light were used, but the results now obtained are sufficiently lower to indicate a lower activity.

Determinations were also made with a portion of the same solution of malic acid but neutralized with potassium hydroxid previous to the addition of uranium acetate. With the tenth-normal solution the results in duplicates were for the incandescent electric light 18.86 and 18.69, respectively, and for sodium light 18.63 and 18.47, respectively, or, computed for a 1 per cent solution, an average of 28.01 and 27.67, respectively, for the two kinds of light. A fifth-normal solution of malic acid not treated with uranium acetate in a 400 mm tube gave a reading 0.46, equivalent to 0.17 for a 1 per cent solution in a 200 mm tube. Subtracting this activity of the free malic

[1] J. Ind. Eng. Chem., 1911, *3:* 564. [2] Ibid., p. 565, Table I. [3] Ibid., p. 568, Table IV.

acid from that found for the uranium compound, we have as the increase due to uranium for a 1 per cent solution of the unneutralized acid for the incandescent light 28.34, giving us a malic acid factor of 0.0353, and for yellow sodium light 28.12, giving the factor 0.0356. If we take the values obtained with the neutralized malic acid we have for the incandescent electric light an increase of 27.89, giving a factor of 0.0359, or for sodium light 27.50, giving the factor 0.0364. It would be desirable to try preparations of malic acid from other sources or purify a sample to redetermine the activity of the uranium compound and the correct factor.

F. F. Fitzgerald: My experience has shown that the use of bromin sometimes seriously affects the rotation.

DISCUSSION.

The comparative simplicity of the procedure leads one to expect well agreeing results on the part of different workers. This expectation is amply realized. A study of the method shows that probably it can be materially improved both in ease of operation and in accuracy of results. In its present form, however, it is capable of giving results of value in detecting addition of fruit products containing malic acid to those in which it is normally absent. The method fails when it is tried with fruit products containing material amounts of color or tartaric acid.

The use of bromin in decolorizing, in the light of the experience of Johnson and Fitzgerald, seems not well advised. Efforts have been made, therefore, by your associate referee to find a general method applicable to a wide range of fruit products. The barium acetate method developed by Yoder [1] for the determination by use of uranium acetate of malates in cane and maple products, with such changes as make it generally applicable to food products, appears to be of great promise in this direction. As not only the original procedure but the more important of the changes adapting the method generally to fruit products have been suggested by Mr. Yoder, your associate referee suggests that the procedure be known as the Yoder method. It is here described:

YODER METHOD FOR THE ESTIMATION OF MALIC AND TARTARIC ACIDS.

Add to 25 cc or 25 grams of sample sufficient alcoholic tenth-normal hydrochloric acid to decompose organic acid salts of the alkaline earth bases. (Twice as much hydrochloric acid as is necessary to neutralize the ash would be sufficient.) Add several volumes of 95 per cent alcohol, stir well, and, when the precipitated pectins have settled somewhat, filter, using Büchner funnel with suction.[2] Wash with 95 per cent alcohol and transfer filtrate and washings to a 400 cc beaker. Add sufficient alcoholic tenth-normal alkali to neutralize the mineral acid added and the free acid present in the sample, and add 5 cc of a solution of barium acetate containing 5 grams in 100 cc.

Make up about 375 cc with 95 per cent alcohol and digest on the steam bath for about one hour, when the precipitated barium salts of the organic acids will be found to have settled well and to have become somewhat granular and the supernatant liquors to have become clear.

Collect the precipitate, using gentle suction on a Büchner funnel, employing a filter about 2 cm wider than the interior diameter of the funnel, with the edges projecting upward so as to form a shallow cell. Wash with 95 per cent alcohol. Remove the precipitate and paper and dry by placing on a watch crystal on the steam bath. Transfer the precipitate to a flask, using black paper, spatula, and brush, and thoroughly extract the filter paper with successive portions of hot water, adding the washings to the flask. Add water to the flask nearly to the mark and digest on the steam bath to promote the complete solution of the barium malate. The amount of water used must be large enough to dissolve the barium malate completely. At room temperature 100 cc of water dissolve about 0.9 gram of barium malate,[3] and about 0.030 gram of barium tartrate.[4] There is marked tendency for these salts,

[1] J. Ind. Eng. Chem., 1911, *3:* 572.

[2] Gelatinous alcoholic precipitates are best filtered and washed by using suction, as the jellies then usually collapse on the filter, releasing liquid which otherwise would be removed but slowly.

[3] Cantoni and Basadona, Bull. soc. chim., 1906 (3), *35:* 730.

[4] Cantoni and Zachodev, Bull. soc. chim., 1905 (3), *33:* 751.

at least the malate, to form supersaturated solutions. Cool, make up to the mark, and if tartaric acid is present allow the solution to stand overnight so that the barium tartrate dissolved in the hot water may have opportunity to separate in case there is marked tendency to form a supersaturated solution.

For malic acid, filter and polarize a portion of the filtrate. Add to 20 cc of filtrate sufficient solution of uranium acetate containing 10 grams in 100 cc to provide about 1½ molecular equivalents of uranium (see p. 64) and ½ cc of glacial acetic acid (see p. 68), make up to 25 cc, and polarize. Correct the polarization for dilution by increasing by one-fourth. The difference between this result and that before the addition of uranium salt times the Yoder factor 0.0338 gives the malic acid present in terms of grams per 100 cc. If tartaric acid is present, it is necessary to add to the polarization a correction to be determined experimentally to compensate for the tartaric acid present.

For tartaric acid wash the residue on the filter with a little cold water and transfer to a flask or beaker and treat with sufficient sodium sulphate to decompose the barium tartrate. Digest for a time on the steam bath in order that the precipitated barium sulphate may become granular, filter and wash, and then concentrate, if desired, finally bringing up to a known volume. Polarize before and after addition of uranium acetate as in the procedure for malic acid. The Yoder factor for tartaric acid is 0.0397. Finally correct by increasing the amount of tartaric acid found by the amount held in solution in the water used in dissolving the barium malate to be determined experimentally.

Alcoholic tenth-normal acid and alkali are used to avoid dilution of the sample with water before precipitating the organic acids in strong alcoholic solution by the addition of barium acetate. The barium tartrate dissolved in the water used in dissolving the malic acid evidently decreases the negative reading due to malic acid; the necessary correction is still to be determined experimentally.

This general method is quite long. It is anticipated that in grape products all of the steps except possibly the original acidification with mineral acid will have to be taken. With products free from tartaric acid and containing little color, such as vinegars other than wine vinegar, fruit juices, jellies and sirups, cane and maple products, it is probable that the precipitation as barium salts may be omitted. Precipitation as barium salts probably will allow the determination of malic acid in colored products by the use of uranium acetate, as it is observed that a large proportion of the color remains in solution in the alcohol, and in some instances is carried down in the alcohol precipitate.

INFLUENCE OF VARYING AMOUNTS OF URANIUM ACETATE ON THE OPTICAL ROTATION OF MALIC AND TARTARIC ACIDS.

In attempting to attain the maximum rotations observed by Yoder in his original papers, difficulty was met with in finding the correct conditions. The polarizations with tartaric acid were especially low. Further study of the problem has led to a study of the effect of uranium acetate when added in varying amounts to solutions of malic acid and tartaric acid, with the result that the failure to secure the higher polarizations is explained by the remarkable facts developed.

Solutions of malic and tartaric acids with uranium acetate were prepared in such a way that when the volume was finally completed for polarization each solution contained its respective acid in the proportion of 1 gram in 100 cc at 20° C.

The amounts of uranium salt used ranged from 0.1 to 2.5 molecular equivalents of uranium for each molecular equivalent of the two respective acids. The amount of uranium present in the uranium acetate used (Mallinckrodt's preparation "free from sodium" was used) was determined by igniting to U_3O_8. The percentage found was 55.475, theoretical for $UO_2(C_2H_3O_2)_2.2H_2O$, 56.18.

From the molecular weights it is calculated that each molecular equivalent of malic or tartaric acid requires 430 grams of the preparation of uranium acetate at hand. Each gram, therefore, of the respective acids requires 3.2090 and 2.8665 of the uranium acetate for one equivalent or 32.09 cc and 28.665 cc of solution of uranium

acetate containing 10 grams in 100 cc. This solution was easily prepared by dissolving in warm water, cooling, making to the mark, and filtering.

The solutions described below were prepared by adding measured amounts of the solution of uranium acetate (about 25 cc portions at 20° C.) at room temperature to solutions of malic and tartaric acids, both free and neutralized with soda containing 2 grams in 100 cc, finally making up at 20° C. to 50 cc, except where noted in the tables. Polarizations were made at 20° C.

Acid solutions containing uranium acetate.

Acid and No.	Uranium acetate added.		Polarizations, °V.	Acid and No.	Uranium acetate added.		Polarizations, °V.
	Molecular equivalent.	Cubic centimeters of solution containing 10 grams in 100 cc.			Molecular equivalent.	Cubic centimeters of solution containing 10 grams in 100 cc.	
Free malic acid:				Free tartaric acid:			
1	None.	None.	− 0.20	1	None.	None.	+ 0.80
2	1/10	1.60	− 1.70	2	1/10	1.43	+ 1.45
3	1/5	3.21	− 3.70	3	1/5	2.87	+ 2.90
4	1/4	4.01	− 4.50	4	1/4	3.58	+ 3.55
5	1/3	5.35	− 6.20	5	1/3	4.78	+ 4.90
6	1/2	8.02	− 9.45	6	1/2	7.17	+ 7.65
7	2/3	10.70	−12.30	7	2/3	9.56	+10.45
8	1	16.045	−18.75	8	1	14.33	+15.85
9	1¼	20.06	−23.05	9	1¼	17.91	+19.30
10	1½	24.07	−27.00	10	1½	21.50	+21.60
Neutralized malic acid:				Neutralized tartaric acid:			
1	None.	None.	− .65	11	None.	None.	+ 2.25
2	1/10	1.60	− 3.60	12	1/10	1.43	+ 4.85
3	1/5	3.21	− 6.50	13	1/5	2.87	+ 7.20
4	1/4	4.01	− 8.00	14	1/4	3.58	+ 8.45
5	1/3	5.35	−10.60	15	1/3	4.78	+10.65
6	1/2	8.02	−15.50	16	1/2	7.17	+14.65
7	2/3	10.70	−20.10	17	2/3	9.56	+18.65
8	1	16.045	−29.40	18	1	14.33	+24.90
9	1¼	20.06	−29.45	19	1½	17.91	+24.30
10	1½	24.07	−29.50	20	1½	21.50	+23.25

Yoder [1] found that the addition of moderate amounts of acetic acid increased markedly the rotation of the malic acid uranium samples, larger amounts causing a decrease.

A series of determinations was therefore made to determine the effect of acetic acid in increasing the rotation of neutralized malic acid and tartaric acids. The solutions were made up in the manner just described. The data are shown in the following table:

[1] J. Ind. Eng. Chem., 1911, *3*: 563.

Acid solutions containing uranium acetate and acetic acid.

Acid and No.	Uranium acetate added.		Acetic acid added.	Final volume.	Readings, °V.	Acid and No.	Uranium acetate added.		Acetic acid added.	Final volume.	Readings, °V.
	Molecular equivalents.	Cubic centimeters of solution containing 10 grams in 100.					Molecular equivalents.	Cubic centimeters of solution containing 10 grams in 100.			
Neutralized malic acid:			Cc.	Cc.		Neutralized tartaric acid:			Cc.	Cc.	
1...	1	16.045	None.	.....	−29.4	13...	1	14.33	None.	50	+25.0
2...	1	16.045	½	50	−28.75	14...	1	14.33	½	50	+24.75
3...	1	16.045	1	50	−28.1	15...	1	14.33	1	50	+23.55
4...	1	16.045	2	50	−26.4	16...	1	14.33	2	50	+20.9
5...	1¼	20.06	None.	50	−29.5	17...	1¼	17.91	None.	50	+24.75
6...	1¼	20.06	½	50	−29.95	18...	1¼	17.91	½	50	+25.50
7...	1¼	20.06	1	50	−29.5	19...	1¼	17.91	1	50	+25.1
8...	1¼	20.06	2	50	−28.3	20...	1¼	17 91	2	50	+23.3
9...	1½	24.07	None.	50	−29.15	21...	1½	21.50	None.	50	+23.45
10...	1½	24.07	½	50	−29.75	22...	1½	21.50	½	50	+25.2
11...	1½	24.07	1	50	−29.50	23...	1½	21.50	1	50	+25.05
12...	1½	24.07	2	50.9	[1] −28.9	24...	1½	21.50	2	50	+23.85

[1] Average reading was 28.3. This was increased by 1.8 per cent to compute to volume of 50 cc.

Where but 1 equivalent of uranium acetate was used, the effect of acetic acid was to lower the readings in case of each acid; where 1¼ equivalents were present the result was to increase the reading markedly in case of the neutralized salts of both acids, larger amounts of acetic acid than the quantity giving the maximum causing a lowering. Where 1½ equivalents of uranium acetate were present the maximum reached in each instance was not so high as when but 1¼ equivalents were used. Marked increase, however, in rotation occurs.

To extend the study to include still larger proportions of uranium acetate and to determine the effect of acetic acid in causing the maximum rotation to be developed in the presence of marked excess of uranium salt, the series of observations recorded in the following table was made, using as high as 2½ equivalents of uranium acetate. Here 10 cc portions of solutions of malic and tartaric acids both free and neutralized, containing 5 grams in 100 cc at 20° C., were used, being diluted as before at 20° C. to a final volume of 50 cc, except where otherwise noted in the table.

Solutions containing as high as 2½ equivalents of uranium acetate.

Acid and No.	Uranium acetate added.		Glacial acetic acid used.	Final volume.	Polarizations.	Polarizations corrected to 50 cc.
	Molecular equivalent.	Cubic centimeters of solution containing 10 grams in 100 cc.				
Free malic acid:			cc.	cc.	° V.	° V.
1	1½	24.07	None.	50	.....	−26.9
2	2	32.09	None.	50	.....	−29.1
3	2½	40.11	None.	50	.....	−29.0
Neutralized malic acid:						
4	1½	24.07	None.	50	.....	−29.3
5	2	32.09	None.	50	.....	−29.0
11	2	32.09	½	50.1	+29.7	−29.75
12	2	32.09	1	50.6	+29.35	−29.6
13	2	32.09	1½	50.9	+28.9	−29.4
13a	2	32.09	2	51.6	+28.15	−29.05
Free tartaric acid:						
6	1½	21.50	None.	50	.....	+21.85
7	2	28.66	None.	50	.....	+21.95
8	2½	35.83	None.	50	.....	+20.0
Neutralized tartaric acid:						
9	1½	21.50	None.	50	.....	+23.65
10	2	28.66	None.	50	.....	+21.4
14	2	28.66	½	50	.....	+23.95
15	2	28.66	1	50	.....	+24.5
16	2	28.66	1½	50	.....	+24.4
16a	2	28.66	2	50	.....	+24.0

Depressions in the rotations with increasing amounts of uranium acetate occurred. With free malic acid the tendency is but slight; in the case of tartaric acid, very marked. A similar reduction in rotation of the neutralized salts is also evident. Addition of acetic acid, however, as in the preceding instances, causes the rotation to increase nearly to the same maximum secured where less uranium salt was used.

The results secured here, where the amounts of uranium were the same as used before, are slightly different and perhaps less accurate on account of the greater error incident to measuring the smaller volumes of malic and tartaric acids used. The concentrations of acid used, it should be stated, were standardized against tenth-normal alkali, using phenolphthalein as indicator.

INFLUENCE OF URANIUM ACETATE ON THE POLARIZATION OF SUGARS AND EFFECT OF SUGARS ON THE POLARIZATION OF THE MALIC-ACID-URANIUM COMPLEX.

One other point which it is necessary to touch upon is the influence of the uranium acetate upon the polarization of sugars. Yoder [1] found no effect up to concentrations of 3 grams per 100 cc. Dunbar and Bacon reported marked lowerings of the rotation of both cane and invert sugar. The effect observed by them was, however, due wholly to the increase in volume on adding solid uranium acetate. Tests by your associate referee on this point indicate that there is practically no effect either of uranium acetate upon the rotation of sugars or of the sugars upon the rotation of the malic-acid-uranium complex. In testing out the effect of uranium acetate on the optical rotation of sugar solutions, solutions containing normal weights of sucrose and invert sugar sirup [2] were prepared. Twenty cubic centimeter portions of each were diluted to 25 cc and polarized and 20 cc portions of each were treated with portions of 2 grams of uranium

[1] J. Ind. Eng. Chem., 1911, *3*: 563.

[2] Prepared in the Physical Chemistry Laboratory of the Bureau of Chemistry from cane sugar by the use of invertase.

acetate, made up to 25 cc, and polarized. The work was done at 20° C. in 20 cm tubes. The readings were as follows:

Solution of sucrose, no uranium acetate, +79.75° V.

Solution of sucrose, with uranium acetate, +79.85° V.

Solution of invert sugar, no uranium acetate, −14.2° V.

Solution of invert sugar, with uranium acetate, −13.85° V.

The effect on sucrose is thus within the error of polarization ±0.1° V., while the effect on invert sugar appears to be slightly greater than this.

In a series of trials to determine the effect of presence of sugars on the polarizations, the following solutions were prepared:

1. A solution of malic acid containing 1 gram in 100 cc.

2. Solution containing a normal weight of sucrose and 0.5 gram of malic acid in 100 cc.

3. Solution containing a normal weight of invert sugar sirup and 0.5 gram of malic acid in 100 cc.

Twenty cubic centimeter portions of each of these solutions were diluted to 25 cc and polarized, and 20 cc portions were each treated with 2.1 grams of uranium acetate, then diluted to 25 cc and polarized. As in the study described above, the dilutions and polarizations were made at 20° C. in 20 cm tubes. The results are as follows:

The tube containing the solution of malic acid polarized at −0.10° V. and that containing the solution of malic acid and uranium acetate polarized at −11°, a change in rotation of 10.95°. The tube containing sucrose and malic acid polarized at +79.5° V. and that containing the solution of sucrose and malic acid plus uranium salt polarized at +68.6° V., a change in rotation of 10.9° V., indicating practically no effect of sucrose on the rotation of the malic-acid-uranium complex. The tube containing invert sugar and malic acid in solution polarized at −14.7° V. and that containing invert sugar and malic acid in solution plus uranium salt polarized at −25.7° V., a change in rotation of 11° V., thus showing that invert sugar does not affect perceptibly the polarization of the malic-acid-uranium complex.

Summary.

DETERMINATION OF CITRIC ACID.

(a) The cooperative work on the Pratt method for the determination of citric acid shows that satisfactory duplicates are difficult to obtain, owing probably to the fact that slight changes in the conditions under which the oxidation of the citric acid is accomplished influence largely the yield of acetone. The conditions under which the oxidation is conducted should receive further attention.

(b) The interference of tartaric acid when present in large amounts is demonstrated; the practical noninterference of aconitic acid is probable, but has not been conclusively determined.

DETERMINATION OF MALIC ACID.

(a) Well agreeing results were secured in the cooperative work.

(b) The use of bromin in destroying color was shown to be of doubtful value, as the results were at times rendered inexact.

(c) A description of a general procedure for the estimation of malic and tartaric acids to be known as the Yoder method is given in tentative form, the details to be perfected during the coming year.

(d) A study was made of the optical rotation of malic and tartaric acids, respectively, in the presence of amounts of uranium acetate varying from 0.1 to 2.5 molecular equivalents of uranium and using the respective acids both free and neutralized in the different experiments. Up to 1 equivalent the polarization increased directly with the increase of uranium salt, reaching in the case of the neutralized acids nearly

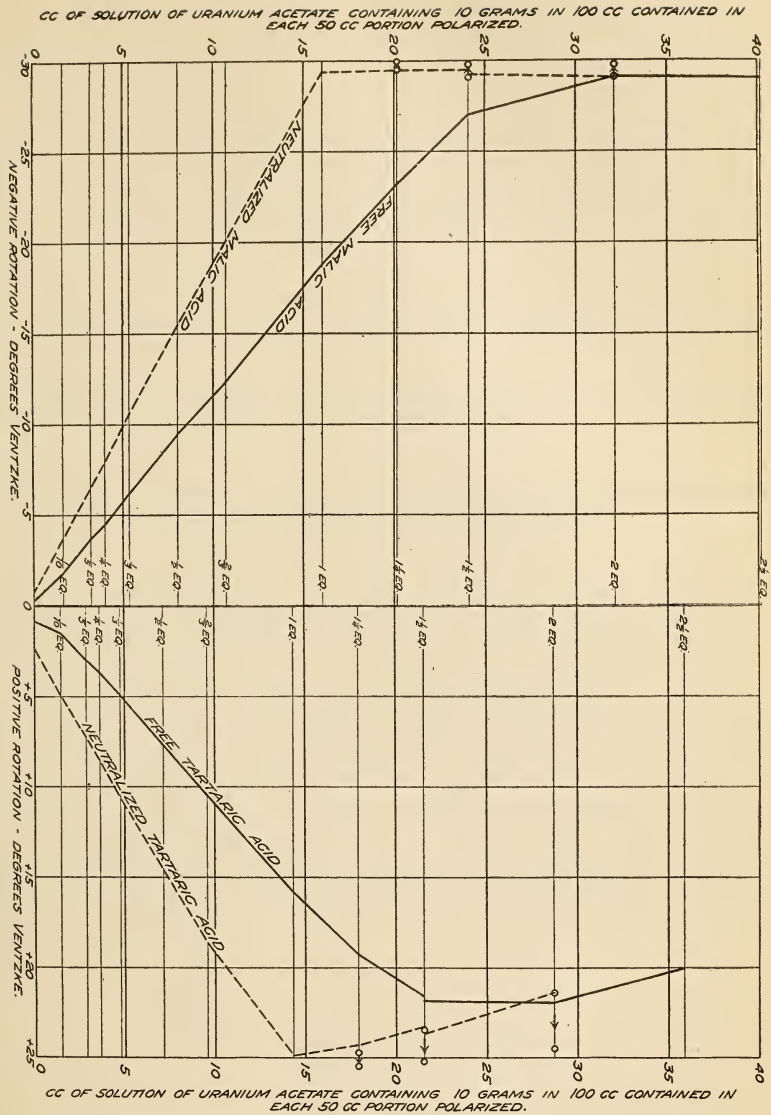


FIG. 2.—Effect on the optical rotation of malic and tartaric acids, respectively (both free and neutralized), of amounts of uranium acetate from $\frac{1}{16}$ to $2\frac{1}{2}$ molecular equivalents and the effect on the optical rotations of the neutralized acids in the presence of uranium acetate, of optimum amounts of acetic acid. 0⟵0 and 0⟶0 indicate such increases in case of malic and tartaric acids, respectively.

a maximum at this point. With the free acids maximum readings were not reached until about 1.75, and 1.5 equivalents of uranium salt were added to solutions of malic and tartaric acids, respectively, and then the maximum readings were lower than in the case of the neutralized acids; particularly was this true with tartaric acid. In the case of free tartaric acid there was found a marked decline in optical rotation upon the addition of larger amounts of uranium acetate than sufficient to give maximum effects. The salts of the two respective acids with uranium acetate also suffered a decline in optical rotation when an excess of uranium acetate was added. In this instance, however, it was found that the addition of acetic acid increased the reading nearly to the maximum. These results taken as a whole indicate that the correct procedure in using the uranium-acetate method is to add uranium acetate to solutions of the neutralized acids, in the presence of sufficient acetic acid to give maximum readings.

(e) No influence of uranium acetate on the polarization of sucrose and invert sugar, respectively, was found, nor of sucrose or invert sugar on the polarization of the malic-acid-uranium complex.

No reports on distilled liquors, beer, and wine were presented. The following paper was read by Mr. Hartmann:

A PROPOSED METHOD FOR THE DETERMINATION OF TARTARIC ACID CONTENT IN WINES AND GRAPE JUICES.

By B. G. Hartmann and J. R. Eoff, Jr.

A method for the determination of the total tartaric acid content in wines is given on page 86 of Bureau of Chemistry Bulletin 107, Revised, under "Tartaric Acid and Tartrates." This method is so well known and has been so generally accepted in enological work that it is hardly necessary to describe it in detail; it will suffice to say that by it the tartaric acid in both the free and combined states is precipitated as potassium acid tartrate and ultimately titrated. That the method gives fairly good results in all cases where free tartaric acid is absent has been demonstrated by the authors. They have, however, found that it is unreliable where large amounts of free tartaric acid are present in the liquid. In order to demonstrate this point, the method was applied to numerous synthetic solutions of known content, with the result that it yielded about 70 per cent of the actual free tartaric acid present. For the sake of brevity only a few results are given in the following table. Solutions 1 and 2 contain free tartaric acid only; Solutions 3 and 4 contain potassium acid tartrate only.

Results on tartaric acid in synthetic solutions of known content.

Solution No.	Tartaric acid contained.	Tartaric acid found.	Tartaric acid recovered.
	Grams.	*Grams.*	*Per cent.*
1	0.994	0.698	70.0
2	.497	.357	71.8
3	.239	.234	97.9
4	.239	.233	97.5

This discrepancy is to be attributed to the fact that the principal reaction in the procedure is reversible, potassium chlorid and tartaric acid giving potassium acid tartrate and free hydrochloric acid, an equilibrium taking place among all the members of the equation, and thus withdrawing an appreciable amount of tartaric acid from the precipitation. The amount of tartaric acid lost to the precipitation is practically

dependent upon the percentage of free tartaric acid present. To offset the decomposing action of the hydrochloric acid, a solution of potassium acetate is added, whereby potassium chlorid and acetic acid are formed. Upon the addition of this salt the usefulness of the method is in the main dependent, the amount of potassium acetate to be added depending largely upon the amount of hydrochloric acid liberated in the main reaction.

Since, however, the free tartaric acid content of the liquid to be examined is unknown, the amount of potassium acetate to be used would either have to be determined empirically, by using increasing amounts of the salt and accepting that quantity of the salt as correct which admits of the highest percentage of total tartaric acid, or by determining the free tartaric acid approximately and thus fixing the amount of potassium acetate to be added. This method, however, would be too tedious of manipulation to be of value to the wine chemist. Then again, it is rather doubtful if even this procedure would make the method entirely reliable, because even in such a case the disturbing influence of the free hydrochloric acid could not be eliminated altogether.

That the temperature at which the reaction mixture is held for 15 hours is of the utmost consequence was shown by the authors. Duplicate determinations in which temperatures of 17° C. and 5° C. were maintained during the precipitation period showed about a 10 per cent increase in tartaric acid in favor of the lower temperature.

Duplicate determinations of tartaric acid at 17° C. and 5° C.

Experiment No.	Tartaric acid contained.	Tartaric acid found.	Tartaric acid recovered.	Temperature of reaction mixture.
	Grams.	*Grams.*	*Per cent.*	° *C.*
1	0.994	0.774	77.9	5
2	.994	.792	79.7	5
3	.497	.401	80.7	5
4	.497	.405	81.5	5
5	.994	.698	70.2	17
6	.994	.698	70.2	17
7	.497	.354	71.3	17
8	.497	.357	71.8	17

From the foregoing it is evident that the method in Bulletin 107, Revised, is hardly to be accepted as reliable and is still less so when it is applied to grape juices high in free tartaric acid. Realizing the drawbacks of this method, especially when applied to grape juices, the authors endeavored to modify the procedure by eliminating the formation of the objectionable hydrochloric acid. After a great deal of work in this direction, a method suggested itself which has proved entirely satisfactory in every respect.

The proposed method is a modification of the old one, inasmuch as it is also based upon the conversion of tartaric acid into potassium acid tartrate with ultimate titration. In it, however, the formation of the hydrochloric acid is eliminated, thus making the addition of potassium acetate unnecessary. The following is the method in detail:

PROPOSED METHOD FOR THE DETERMINATION OF TOTAL TARTARIC ACID.

Preliminary procedure.—Shake sample thoroughly. Measure 20 cc of the juice into a 250 cc beaker and titrate with tenth-normal sodium hydroxid until 2 drops of the juice when mixed on a porcelain tile with several drops of a neutral azolitmin solution give no red tint. If no more than 25 cc of tenth-normal sodium hydroxid are required for this titration, one may filter the grape juice to be analyzed either through cotton or through paper. Should, however, the titer for 20 cc of juice exceed 25 cc of

tenth-normal sodium hydroxid, then it is advisable to dilute the juice with an equal amount of water; for example, take 500 cc of juice and make to 1 liter. Filter this solution through a large fluted paper, discarding the first 50 cc that pass through.

Determination.—Neutralize 100 cc of the juice (diluted or undiluted as the case may be) exactly with double-normal sodium hydroxid. The amount of alkali necessary for neutralization may be calculated from the acidity of the juice previously ascertained. If the volume of the solution is increased more than 10 per cent by the addition of the double-normal alkali, either evaporate the solution to approximately 100 cc or make provision for this dilution. This may be accomplished by adding for every 10 cc over 100 cc obtained 1.5 grams of potassium chlorid and 2 cc of 95 per cent alcohol. The neutralized solution should never exceed 125 cc, however. To the neutralized juice now add tartaric acid, the amount to be added depending absolutely upon the amount of double-normal sodium hydroxid required to neutralize the 100 cc juice taken, as ascertained from the following equation:

cc 2N alkali required to neutralize 100 cc juice $\times 0.15=$ grams tartaric acid required.

Record weight of tartaric acid added. (Be assured of the purity of the tartaric acid added.) After the tartaric acid is completely dissolved, add 2 cc of glacial acetic acid and 15 grams of potassium chlorid. Stir until the potassium chlorid is dissolved. It will almost invariably occur that potassium acid tartrate begins to form before the potassium chlorid is dissolved, but these salts are easily distinguished from each other. Add now 20 cc of 95 per cent alcohol, stir vigorously for five minutes, and let stand at least 15 hours at a temperature not exceeding 15° C. After this interval decant the solution through either a Gooch crucible with asbestos felt or a Büchner funnel of 7 cm diameter, into which is exactly fitted a strong filter paper and use gentle suction. Wash with solution composed of 100 cc of water, 15 grams of potassium chlorid, and 20 cc of 95 per cent alcohol, making three washings of 7 cc each. With care the precipitate, beaker, and funnel may be thoroughly cleansed of acetic acid with these washings. Transfer with hot water the precipitate and asbestos if the Gooch is used, or paper if Büchner funnel is used, to the original beaker, add about 50 cc of water, bring to a boil, and titrate with either tenth-normal or fifth-normal sodium hydrate, using either litmus solution or phenolphthalein as indicator, preferably phenolphthalein.

The amount of tartaric acid in the 100 cc taken is then found from the following:

$$(X+1.5)\times 0.015-Y=Z,$$

where X=cc of tenth-normal alkali required in titration,
Y=grams of tartaric acid added,
Z=grams of total tartaric acid in the 100 cc taken.

If the diluted juice is taken for the determination, of course, Z would represent but half the total tartaric acid contained in 100 cc of the original juice.

Since pectin bodies and gums which are contained in hot pressed grape juices in large amounts make the washing of the potassium acid tartrate crystals very difficult, the tartaric acid content often is higher than corresponds to the truth. It is necessary, therefore, to remove the pectin bodies before determining the tartaric acid in such juices. This may be accomplished by transferring 100 cc of the juice to a liter evaporating dish and heating on a boiling water bath for half an hour. The water evaporated is replaced from time to time as it evaporates, and after this time 50 cc of water is added, and the whole boiled for a few minutes. Transfer to a 200 cc flask, and after cooling make to volume and filter. Determine the tartaric acid in 100 cc of this solution by neutralizing with double-normal sodium hydrate and adding tartaric acid, etc., as described.

A large number of synthetic solutions containing cream of tartar, free tartaric acid, tartaric acid combined with alkaline earths, and sugar were prepared and analyzed according to the modified method and the method in Bulletin 107, Revised. In all instances the proposed method gave extremely satisfactory results, whereas the old method was much in error.

Determination of tartaric acid by the proposed and provisional methods.

Solution No.	One liter contained—				Tartaric acid.				
	Cream of tartar.	Tartaric acid.	Calcium carbonate.	Cane sugar.	Contained.	Found by proposed method.		Found by old method.	
						I.	II.	I.	II.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams per 100 cc.*	*Grams per 100 cc.*	*Grams per 100 cc.*	*Grams per 100 cc.*	*Grams per 100 cc.*
1	3	1.988	.....	160	0.438	0.427	0.425	0.384	0.382
2	3	3.976	0.7	160	.637	.636	.642	.561	.564
3	3	.....	.....	160	.239	.234	.233	.227	.227
4	.....	19.88	.....	160	1.988	1.914	1.939	1.262	1.282

A very decided advantage of the proposed method over the old method is shown in cases where very low tartaric acid content in the wine is encountered. In such cases the acid potassium tartrate forms slowly and may even refuse to precipitate at all. The addition of the tartaric acid required in the proposed method hastens the precipitation greatly and admits of a more complete reaction. It is hardly necessary to say that the method will not determine less than 0.020 gram of tartaric acid per 100 cc because of the solubility of the potassium acid tartrate under the conditions maintained. In such cases several hundred cubic centimeters of the wine should be evaporated to 100 cc and the method applied on the concentrated solution.

After having determined the usefulness of the proposed method as stated above, it suggested itself to the authors that the addition of the tartaric acid might be omitted and the same advantage derived by half neutralizing the acidity of the wine or juice with normal sodium hydrate and then proceeding with the potassium chlorid, acetic acid, and alcohol additions. A large number of determinations on this basis have proved that such a procedure gives fairly satisfactory results, but somewhat lower than the truth.

Determination of tartaric acid in samples of half neutralized acidity.

Experiment No.	Tartaric acid.		Experiment No.	Tartaric acid.	
	Proposed method.	Half neutralized.		Proposed method.	Half neutralized.
1	0.74	0.68	5	0.72	0.63
2	.54	.50	6	.78	.71
3	.99	.96	7	.80	.77
4	.76	.71			

A PROPOSED METHOD FOR THE DETERMINATION OF MALIC ACID IN GRAPE JUICES.

By B. G. Hartmann and J. R. Eoff, Jr.

The problem of devising a method for the determination of malic acid in grape juice has been very difficult and the literature to-day details no method that can be accepted as giving even concordant results when dealing with juices of the many varieties of grapes. P. B. Dunbar, of the Bureau of Chemistry, has proposed a method for the determination of this acid which is based upon its optical characteristics under certain conditions. It is to be regretted that he has so far been unable to apply his procedure with any great degree of accuracy to other juices than white juices. It occurred to the authors from work they had carried out on the acids of the grape that there was a pos-

sible means of obtaining the free malic acid of a grape juice by subtracting the sum of the free tartaric acid and the acidity due to the acid salts from the total fixed acidity of the juice. In German enological laboratories such a procedure has been tried and the results indicated by their formulas are of such a nature as to add encouragement to the idea. Elaborating upon their beginning the following formula was devised to indicate the free malic acid in a grape juice:

$[A-[(B\times 0.0075)+C]]\times 0.8934$=free acids, other than tartaric (which we express and hereafter call "free malic acid").

Wherein A=fixed acidity of juice expressed as grams of tartaric acid per 100 cc of juice.

B=total alkalinity of ash.

C=free tartaric acid as grams per 100 cc of juice.

Having thus obtained the free malic acid, it devolved upon us to ascertain the amount, if any, of malic acid combined with the bases occurring in a grape juice. Without entering at this time into the discussion of our arrival at the following expressions for the combinations of the various acids and bases contained in grape juices, we give the following as a means for determining these combinations:

The factors necessary for the calculation of the combinations between acids and bases are:

A=the cubic centimeters of tenth-normal acid equivalent to one-half of the total tartaric acid contained in 100 cc of juice.

B=the total alkalinity of the ash.

C=the alkalinity of the water-soluble ash.

D=the alkalinity of the water-insoluble ash.

Then, if found by analysis that—

(1) A is greater than B:

Cream of tartar	$=0.0188\times C$
Free tartaric acid	$=.015\times(A-B)$
Tartaric acid to alkaline earths	$=.015\times D$
Malic acid to potash	$=.0$
Malic acid to alkaline earths	$=.0$

(2) A equals B or smaller than B but greater than C:

Cream of tartar	$=0.0188\times C$
Free tartaric acid	$=.0$
Tartaric acid to alkaline earths	$=.015\times(A-C)$
Malic acid to potash	$=.0$
Malic acid to alkaline earths	$=.0134\times(D-(A-C))$

(3) A is smaller than C:

Cream of tartar	$=0.0188\times A$
Free tartaric acid	$=.0$
Tartaric acid to alkaline earths	$=.0$
Malic acid to potash	$=.0134\times(C-A)$
Malic acid to alkaline earths	$=.0134\times D$

Having by this means determined the amount of malic acid existing in a juice as acid salts of potassium or the alkaline earths or both, it is necessary only to calculate the equivalents of malic acid represented and add this figure to that already determined of free malic acid to arrive at the total malic acid content.

Since the details of the methods of arriving at the factors necessary for the above calculations (acidity, total tartaric acid, and the alkalinities of the ash) have been found by us to be imperfectly known, we deem it advisable here to give briefly those details of manipulation which we have demonstrated to our satisfaction as being capable of yielding the best checks in the hands of different chemists.

The details of the tartaric acid determination will be found on pages 72 to 73.

ACIDITY OF THE JUICE.

Measure 20 cc of the original or diluted juice[1] into a beaker and titrate with tenth-normal sodium hydroxid. Use a neutral azolitmin solution as an outside indicator. A tile is spotted with the azolitmin solution and the solution undergoing titration is spotted into the azolitmin until two drops do not change the shade of the azolitmin. The acidity is usually expressed as grams of tartaric acid per 100 cc; 1 cc of tenth-normal alkali is equivalent to 0.0075 gram of tartaric acid.

ALKALINITIES OF THE ASH.

1. *Ash.*—Measure into a platinum dish 25 cc of the original juice or 50 cc of the diluted juice, evaporate on a water bath to thick sirup, and ash carefully at dull red heat, never allowing the fumes to ignite. If difficulty is experienced in burning the ash white, leach with hot water. To the white ash add about 5 cc of water and 3 drops of a 10 per cent solution of ammonium carbonate. Evaporate and heat below redness to expel ammonium salts. To the ash add hot water; police the whole, transfer with hot water to a 7 cm filter, and there wash successively with hot water until the filtrate amounts to 50 cc. This filtrate will be called the water-soluble ash solution. The paper and residue are returned to the dish and gently ignited, thus obtaining the water-insoluble ash portion.

2. *Alkalinity of the water-soluble ash.*—To the water-soluble ash solution add an excess of tenth-normal sulphuric or hydrochloric acid, bring the solution to a boil, and titrate back with tenth-normal sodium hydroxid, using phenolphthalein as indicator. The difference between the cubic centimeters of acid and the cubic centimeters of alkali required to titrate back is the alkalinity of the water-soluble ash. This is to be calculated to 100 cc of the original juice.

3. *Alkalinity of the water-insoluble ash.*—To the water-insoluble ash portion in the dish add about 10 cc of water and then an excess of tenth-normal hydrochloric acid or sulphuric acid, bring the whole to a boil, being careful to heat only the bottom of the dish. When solution is complete, titrate back, in the dish, with tenth-normal alkali. The alkalinity of the water-insoluble ash is calculated in the same manner as that of the water-soluble ash.

It is more than probable that chemists familiar with wine or fruit-juice analysis may take exception to the above outlined method of arriving at the free malic acid content of a grape juice, upon the ground that the figure obtained by the formula on page 75, does not represent truly the free malic acid, but rather all the free fixed acids other than tartaric. Undoubtedly exception will also be made to the manner of arrival at the combined malic acid content, upon the ground that the laws of chemical equilibrium will not admit of such an assumption. These possible criticisms have been recognized. In view of this we desire to state, as forcibly as we can, that there is no claim made for absolute accuracy, nor do we intend to imply by the use of the name "malic acid" that this is the actual malic acid present. Undoubtedly there are other acids present that would be included in the figure we obtain and term "malic acid." We, however, wish to say that the method will give without doubt a figure that is constant within the range of the ordinary processes of chemical analysis; more so than any other method for determining this constituent that has come within our observation, and when one considers that the bulk of the acid in a grape juice is indisputably malic acid, after the elimination of the tartaric acid, we claim that we are justified in a measure in naming the result "malic acid."

We have made many experiments to substantiate our conclusions upon this matter, and have selected from among them the following results as illustrative of the correctness of the assumption.

[1] See p. 72: Proposed method for the determination of tartaric acid.

Determination of malic acid by proposed method.

Determination.	Solution I.	Solution II.	Solution III.	Determination.	Solution I.	Solution II.	Solution III.
	Grams per 100 cc.	*Grams per 100 cc.*	*Grams per 100 cc.*		*Grams per 100 cc.*	*Grams per 100 cc.*	*Grams per 100 cc.*
Potassium acid tartrate:				Malic acid to earth alkalics:[1]			
Contained	0.40	0.30	0.20	Contained	0.00	0.03	0.17
Found	.33	.26	.19	Found	.00	.00	.16
Free tartaric acid:				Free malic acid:[1]			
Contained	.60	.00	.00	Contained	1.12	.67	.25
Found	.68	.00	.00	Found	1.08	.69	.27
Tartaric acid to earth alkalics:				Total acid as tartaric:			
Contained	.18	.09	.00	Contained	1.968	.850	.495
Found	.16	.11	.00	Found	1.969	.851	.488
Malic acid to potash:[1]				Total tartaric acid:			
Contained	.00	.00	.16	Contained	1.100	.327	.158
Found	.00	.00	.13	Found	1.106	.324	.152

[1] Expressed as tartaric.

Determination of malic acid in grape juice by proposed method.

Determination.	Analysis by Johnson.	Analysis by Dunbar.	Analysis by Hartmann and Eoff.	Determination.	Analysis by Johnson.	Analysis by Dunbar.	Analysis by Hartmann and Eoff.
	Grams per 100 cc.	*Grams per 100 cc.*	*Grams per 100 cc.*		*Grams per 100 cc.*	*Grams per 100 cc.*	*Grams per 100 cc.*
Acidity of the juice as tartaric	1.04	1.06	1.02	Cream of tartar	0.64	0.68	0.59
Total tartaric acid	.51	.54	.47	Free tartaric acid	.00	.00	.00
Total alkalinity of ash, cc tenth-normal alkali per 100 cc	48.6	46.2	43.1	Tartaric acid to alkaline earths	.00	.00	.00
Alkalinity of water-soluble ash, tenth-normal alkali per 100 cc	42.6	41.8	36.5	Malic acid to potassium expressed as tartaric	.13	.09	.08
Alkalinity of water-insoluble ash, tenth-normal alkali per 100 cc	6.0	4.4	6.6	Malic acid to alkaline earths expressed as tartaric	.09	.07	.10
				Free malic acid	.61	.63	.62
				Total malic acid	.81	.75	.78

REPORT ON VINEGAR.

By W. A. Bender, *Associate Referee.*[1]

In pursuance of the recommendation made in 1911, several methods as then submitted, and one new one, were selected for study and cooperative work. Two samples, one of pure generator cider vinegar made in the presence of the referee in the winter of 1911 and since then stored in wood, and one containing 50 mg of formic acid per 100 cc, were sent to the 10 collaborators. Reports were received from seven, and the referee wishes here to thank them for the careful way in which they performed the work. The following instructions accompanied the samples:

Directions for Cooperative Work on Vinegar.

Reducing sugars before inversion after evaporation.—(1) Evaporate 10 cc to dryness on the steam bath. Proceed according to Munson and Walker's method, Bulletin 107, Revised, page 241, transferring the solids to the alkaline copper solution with the aid of 50 cc of water.

[1] Read by L. M. Tolman.

(2) Evaporate 50 cc to 5 cc on the steam bath; add 25 cc of water and evaporate to 5 cc. Transfer to a volumetric flask, make up to the mark and proceed according to Munson and Walker's method, using a quantity equivalent to 10 cc of sample.

(3) Proceed as in (2), but after the second evaporation again add 25 cc of water and evaporate to 5 cc, making three evaporations in all.

(4) Proceed as in (2), but make three additions of 25 cc of water, making four evaporations in all.

Polarization.—Add to 50 cc of sample 5 cc of lead subacetate solution, shake and let stand 30 minutes. Filter through a double filter until clear and polarize in a 200 mm tube, adding a correction of 10 per cent for dilution. (Note: Please adjust the polariscope carefully before making this reading, as a difference of 0.2° or 0.3° makes a large percentage error.)

Solids.—Measure 10 cc of filtered vinegar into a tared flat-bottom platinum dish of 50 to 60 mm diameter, evaporate on the steam bath to a thick sirup, and dry for exactly two and one-half hours in the water oven at the temperature of boiling water; cool and weigh. It is essential to use a flat-bottom dish. (Note: The object of this experiment is to determine whether this method will give concordant results in the hands of different analysts if carefully followed. The referee therefore suggests the following precautions: Do not use a dish that is over or under size or one that sags in the middle. Do not allow the solids to remain on the steam bath longer than is necessary to bring them to a thick sirup that does not run; that is, just barely to dryness. Do not exceed the two and one-half hour limit by more than five minutes. Kindly report the diameter of the bottom of the dish used.)

Formic acid.—(Fincke's method, Zts. Nahr. Genussm., 1911, *21:* 1; 1911, *22:* 88.) Add 0.4 to 0.5 gram of tartaric acid to 100 cc of sample and distill with steam. Pass the outflowing steam through a boiling mixture of 15 grams of calcium carbonate and 100 cc of water and keep this volume constant throughout the process. Collect 1,000 cc of distillate and reduce the volume of sample to 30 to 40 cc. Discard the distillate. Filter and wash the calcium carbonate mixture, make faintly acid with hydrochloric acid, add 10 to 15 cc of mercuric chlorid solution (10 grams of mercuric chlorid and 3 grams of sodium chlorid to 100 cc), and heat in a boiling water bath for two hours. Filter on a tared Gooch crucible and wash with water, alcohol, and finally ether. Dry and weigh as mercurous chlorid. The factor for formic acid is 0.0975.

(Apparatus and precautions: The apparatus may be set up as in the case of the ordinary steam distillation, with certain additions. A liter Jena erlenmeyer, containing a few small pieces of submerged pumice stone to insure steady boiling, serves as a steam generator. The steam is led from this into a 300 cc round-bottom boiling flask, preferably with a short neck, containing the sample to be tested. The delivery tube from this flask is of rather large size and leads into a liter Jena pear-shaped nitrogen flask cut off so that the neck is about 3 inches long. This flask contains the carbonate mix; the bottom of the entry tube leading into it should be blown into a bulb and pierced through with 6 or 7 small holes, arranged in a ring a short distance above the bottom, in order to divide the entering stream into as many small bubbles as possible to get good contact with the carbonate mix. From this flask a delivery tube leads to a condenser, simply for convenience and to judge the volume of the distillate. Restrict the volume of filtrate and washings from the carbonate mix to about 140 cc. Evaporate, if necessary, to this volume before acidulating. In heating the reaction mixture, immerse in the bath to the level of the liquid, but not over this level, and filter soon after the heating is completed. The distillation takes from 2 to 2½ hours.)

Alcohol precipitate.—Evaporate 100 cc of sample to 15 cc. When cool add 200 cc of 95 per cent alcohol slowly and with constant stirring. Add the first 50 cc by means of a pipette. Allow the mixture to stand overnight and from this point follow the method as given in Bulletin 107, Revised, p. 80.

Results of Work on Vinegar.

The results received are shown in the following table:

Results of collaborative work on vinegar.

Collaborator.	Reducing sugars before inversion after evaporation.				Polarization.	Solids.	Diameter of dish.	Alcohol precipitate.	On separate sample. Formic acid.
	Evaporation to dryness.	2 evaporations to 5 cc.	3 evaporations to 5 cc.	4 evaporations to 5 cc.					
					° *V.*	*Grams per 100 cc.*	*Mm.*	*Grams per 100 cc.*	*Grams per 100 cc.*
W. B. D. Penniman, Baltimore, Md. (W. W. Randall, analyst)	[1] 0.507	[1] 0.547	[2] 0.535	[1] 0.529	−0.8			0.240	[3] 0.044
S. W. Wiley, Baltimore, Md. (J. F. McKinnell, analyst).	.55	.569	.563	.530	− .8	2.43	73	.357	.03
E. R. Lyman, Portland, Oreg.	[1] .524	[1] .519	[1] .522	[1] .507	−1.1	2.56	50	.31	.047
Henry L. Schulz, Detroit (H. W. Haynes, analyst)..	.528	.525	.540	.535	− .77	2.57		.31	.0556
W. J. McGee, New Orleans, La	[4] .548	[2] .531	[2] .541	[2] .527		2.67 2.66	55		[4] .035
R. W. Balcom, Nashville, Tenn	[1] .54	[1] .55	[1] .54	[1] .54	− .9	2.62 2.62	38	.28	.047
F. L. Shannon, Lansing (Miss Childs, analyst)	[1] .535	[1] .550	[1] .536	[1] .525	−1.87	2.34 2.34	60	[1] .29	[1] .050
W. A. Bender, New York, N. Y.								.30	[4] .053
E. W. Magruder, Richmond, Va. (C. M. Bradbury, analyst) [5]	.49	.48	.49	.48	− .92	2.53	65	.23	[6] .090
Weighted means	.534	.539	.538	.521	− .87	2.53		.297	.045

[1] Average of 2 determinations.
[2] Average of 4 determinations.
[3] Average of 2 determinations (500 cc only of distillate).
[4] Average of 3 determinations.
[5] Results received too late to be included in averages.
[6] The analyst doubts the figure for formic acid.

Comments by Analysts.

W. W. Randall: Solids: Not performed, as we have not the required form of platinum dish. Polarization: Our polariscope is not a high-grade instrument, and the determination of fractions of degrees can not be made with certainty.

J. F. McKinnell: Formic acid: A determination of 500 cc was also tried, but the results gotten were slightly lower than those obtained by taking off 1,000 cc.

E. R. Lyman: Reducing sugars: No difficulties were encountered, and the only comment I have to make is on the sugar determinations. The results by the methods 1, 2, and 3 I consider identical, since the duplicates showed as much variation as the methods themselves. Method 4 seems to indicate a slight loss of volatile reducing material, though I should like to repeat the determination several times before saying positively that this is true. In any case, the difference is so very slight as to be hardly worth the trouble of the repeated evaporations.

W. J. McGee: On the vinegar containing a known amount of formic acid, I made five determinations, with results which varied considerably; in fact, I did not get any two checks which seemed entirely satisfactory to me. The average of the last three determinations was the same as the last determination which I am reporting. My experience is that this train of apparatus puts too heavy a back pressure on the steam generator, so that its working is irregular; in other words, there will be no action until the pressure is enough to overcome the back pressure, when suddenly a large quantity of steam will pass over, throwing up the contents of each flask very high, thus giving a good deal of chance for loss by entrainment. In the last determination I made, I corrected this by applying a very gentle suction at the end of the apparatus, insuring a quiet steady action, and as this result is the average of the last three, I believe it is a modification worthy of consideration.

F. L. Shannon: The only suggestion or criticism that I have to offer would be on the apparatus used in the formic acid determination. We found it necessary to incline the flasks toward the steam generator and to use a bulb tube on the flask carrying the calcium carbonate mixture, otherwise some of the calcium carbonate mixture would splash up into the delivery tube and be carried over into the receiver, thus causing a slight error.

Discussion of Results.

Reducing sugars before inversion after evaporation.—This sample contained about the maximum amount of volatile reducing matter (0.22 gram per 100 cc calculated as invert sugar), so that it offered a good test for the removal of the same by varying lengths of evaporation. The average results by the four methods are very close and show little or no choice between them except as to convenience. More than two evaporations, each to 5 cc, appears to be unnecessary.

Polarization.—The method as now used appears to give fairly concordant results. The referee doubts, however, that it gives the true figure for the polarization, on account of the work reported below on another sample of vinegar.

Solids.—This method evidently requires further study before we can get concordant results from different analysts. The lowest results reported may be accounted for by the large diameters of the dishes used, but variations of 0.1 gram per 100 cc with dishes of practically the same diameter show that this method is still capable of improvement. A larger number of cooperative results must be obtained, however, before any changes can be made.

Alcohol precipitate.—This method appears to give fairly good results in the hands of different analysts. As would appear from some work outlined below, however, it depends in a large degree upon the exact amount of residue left after evaporation.

Formic acid.—Herr H. Fincke, of Cologne, is to be congratulated on devising a method by which 0.05 per cent of formic acid can practically all be recovered from a liquid containing in addition 0.2 per cent of other volatile reducing bodies. While the results show individual variations, due in part, perhaps, to lack of familiarity with the method, the referee considers that an average recovery of 45 mg out of 50 present shows the method to be one of great value.

Recommendations.

It is recommended—

(1) That in method "8. Reducing sugars before inversion, after evaporation," as printed in the 1911 Proceedings, the words "Again add 25 cc of water and evaporate to 5 cc" be omitted, the method then reading as follows: "Evaporate 50 cc to 5 cc on the water bath. Add 25 cc of water and again evaporate to 5 cc. Transfer to a volumetric flask, make up to the mark and proceed as in 7, using a quantity equivalent to 10 or 20 cc of sample"; and that this be considered for adoption as provisional in 1913.

(2) That the method for polarization be studied with reference to whether or not the true polarization is obtained after clarifying with lead.

(3 That Fincke's method for formic acid be studied further during the coming year, with a view to adoption in 1913.

(4) That in addition to the above-mentioned methods the following methods as printed in the 1911 Proceedings be studied further: 6. Solids. 11. Ash. 15. Fixed acid. 16. Volatile acid. 17. Lead precipitate.

(5) That the following methods as printed in the 1911 proceedings be adopted as provisional: 1. Preparation of sample. 2. Calculation of results. 3. Specific gravity. 4. Alcohol. 7. Total reducing matters before inversion. 9. Reducing sugars after inversion. 12. Solubility and alkalinity of soluble ash. 18. Color—brewer's scale.

Notes on Vinegar Methods.

Influence of benzoic acid on the glycerin determination.—Glycerin in a sample of pure vinegar was found by Ross's method to be 0.332 gram per 100 cc. The work was repeated on another portion, to which was added 0.1 per cent of sodium benzoate. Amount of glycerin found, 0.327 gram per 100 cc.

Alcohol precipitate method.—Association sample used throughout.

(1) Nearly to dryness; two precipitations; cold; evaporated 100 cc to about 2 cc and added 125 cc of 95 per cent alcohol; centrifuged after 1 hour; dissolved in 10.5 cc of water and added 125 cc of alcohol, first 50 cc from pipette; stood overnight; filtered; washed with 80 per cent alcohol; weight of precipitate less ash, 0.345 gram.

(2) Nearly to dryness; two precipitations; first hot; same as (1) except that first precipitation was made hot (over steam) with practically boiling alcohol; centrifuged at once; weight of precipitate, 0.315 gram.

(3) Evaporated to 5 cc; two precipitations; cold; same as (1) except that first evaporation was to 5 cc only; weight of precipitate, 0.254 gram.

(4) Evaporated to 10 cc; two precipitations; first hot; same as (2) except that first evaporation was to 10 cc only; weight of precipitate, 0.207 gram.

(5) Evaporated to 15 cc; one precipitation; cold; performed as per method submitted to association in 1911; 200 cc of alcohol used; weight of precipitate, 0.303 gram.

Conclusions: The amount obtained with two precipitations decreases as the amount of water left on the first evaporation increases. Hot alcohol appears to give less than cold. Can see no advantage in use of two precipitations. The method evidently depends entirely on the quantities of residue left and of alcohol used.

Formic acid method.—(Zts. Nahr. Genussm., Fincke, 1911, *21* : 1; 1911, *22* : 88.) A dilute solution of formic acid was prepared and its strength determined by titration. Thirty cubic centimeters were used, with the addition of 3 grams of sodium acetate, 18 cc of mercuric chlorid solution (10 grams per 100 cc), and water to about 110 cc. Heated for two hours in boiling water under air reflux condenser. The mercurous chlorid was filtered and weighed as usual. Amount taken, 0.0811 gram. Amounts found, 0.0821 gram and 0.0820 gram.

The following determinations by Fincke's method were made on the regular association sample, which was made to contain 0.050 gram of formic acid per 100 cc. On the first three trials 500 cc of distillate were collected. These gave 0.041, 0.040, and 0.041 gram per 100 cc. Three more distillations were then made, collecting 1,000 to 1,200 cc of distillate, and the amounts obtained were 0.058, 0.049, and 0.052 gram. It appears necessary, therefore, to distill about 1,000 cc in order to recover all the formic acid present.

Polarization.—A sample of vinegar manufactured in the presence of the referee under the usual commercial conditions in the fall of 1911 and spring of 1912 was found upon analysis to give a plus polarization (+0.7° V.) when clarified in the usual way with lead subacetate. A search for the reason for this apparent abnormality developed the fact that lead subacetate and lead acetate have an influence upon the rotation of malic acid, of which there was an unusual amount present (0.32 gram per 100 cc). This vinegar when treated with boneblack alone polarized −0.44° V. The experiments made showed that the addition of one-tenth volume of subacetate to a solution of malic acid of the same strength as found in the sample, and containing also a similar amount of acetic acid, turned the rotation of the solution about 1.3° V. to the right. With the vinegar in question there is a change of 0.4°+0.7°=1.1° V. to the right.

The addition of one-tenth volume of normal acetate (20 per cent solution) to the same solution of malic acid turned the rotation of the solution about 0.9° V. to the right. In the absence of acetic acid the change was 1.3° V. to the right. But in the absence of acetic acid and with subacetate used, the filtrate, which in this case was alkaline, showed a slight change to the left. It would seem that the true polarization of a vinegar containing any considerable amount of malic acid is not obtained when lead is used to clarify. This might have further bearing in the case of other substances, like maple sirup, which may contain malic acid, and where lead is usually the clarifying agent.

REPORT ON FLAVORING EXTRACTS.

By R. S. Hiltner, *Associate Referee.*[1]

Plan of Work.

The cooperative work on flavoring extracts comprised a study of vanilla and ginger extracts. The primary purpose of the work on vanilla extract was to determine the limits of composition and calculated constants of pure standard commercial products, with the view of comparing the data secured with the results obtained by Winton and Berry in their comprehensive study of extracts [2] made according to the United States Pharmacopœia formula and with the data furnished by the association collaborators last year.

The reliability of Wichmann's test for coumarin [3] was made the subject of special investigation. Such a method, when perfected, will be of great value to the food analyst in determining this frequently used adulterant and for shortening the present provisional method for vanillin in vanilla extracts when coumarin is shown to be absent.

A study was made of the Street-Morrison method for determining the approximate composition of alcoholic extracts of ginger.

Vanilla Extracts.

DESCRIPTION OF SAMPLES.

Seven samples of vanilla extracts were sent to the collaborators. One was adulterated with tonka bean extract and caramel. The others were pure vanilla extracts obtained from reliable manufacturers. Each of these firms gave assurance that the samples were fully up to the standard requirements and furnished the formulas and enough of the manufacturing details to permit of accurate interpretation of results. No coloring matter or other adulterant was used. The following is a schedule of the composition of the six commercial samples:

No. 1. Extract adulterated with tonka bean extract and caramel.

No. 2. Extract made from the best grade of Mexican beans, with a menstruum of sugar sirup, 40 per cent "cologne spirits" and 1 per cent glycerin; macerated for two months and then percolated. The finished product was adjusted to accord with U. S. standard.

No. 3. Extract made from average quality Bourbon beans with sugar and 50 per cent alcohol; macerated for 16 months and then percolated. The finished product was diluted so as to contain the extractive matter of 1 pound of beans per gallon of extract. In terms of the U. S. standard this is equivalent to about 12 grams per 100 cc.

No. 4. Extract made from "run of market" Mexican beans by same process as No. 3, but macerated for about 20 months before percolation. The finished product was diluted to the same degree as No. 3.

No. 5. Extract made from Tahiti beans of average quality, by the so-called "machine process" at a temperature of 100° F., requiring only about 36 hours to complete the manufacture. The menstruum was similar to Nos. 3 and 4. The product was diluted to approximate U. S. standard strength.

No. 6. Extract made from old crop Mexican cuts, with sugar and 45 per cent alcohol. Working formula: 38 pounds of beans to 44 gallons of menstruum, macerated for about four months. This product conforms closely to the requirements of the U. S. standard.

No. 7. Extract made according to the following formula: 29 pounds Tahiti beans, market run as to dryness, 12 pounds prime Bourbon beans, menstruum 40 gallons, consisting of sugar and 60 per cent alcohol; macerated for about one month and then percolated, and finally diluted with water to 50 gallons, equivalent approximately to the U. S. standard.

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[1] Read by E. M. Chace.

[2] U. S. Dept. Agr., Bureau of Chemistry Bul. 152, p. 146.

[3] U. S. Dept. Agr., Bureau of Chemistry Cir. 95.

These six samples, therefore, may be regarded as types of standard commercial extracts, differing mainly as to the kind of vanilla beans used and the composition of the menstrua.

METHODS.

Collaborators were asked to use the following methods in the examination of the seven samples furnished:

1. *Coumarin.*

Test each sample for coumarin by Wichmann's method, as described in the Bureau of Chemistry Circular 95. It is suggested that those who have had no experience with the method make a preliminary test using samples of known composition with and without coumarin.

2. *Vanillin, coumarin, normal lead number, and residual color in the filtrate.*

The determinations are to be made in one weighed portion using 50 grams of the sample.

(a) *Vanillin and coumarin.*—In those samples in which coumarin is detected, determine vanillin, etc., by the Winton, Lott, and Berry method, as described in Bureau of Chemistry Bulletin 137, pages 68 and 120.

(b) *Vanillin.*—In those samples in which coumarin is not detected, follow the same method as under (a), except evaporate directly the first ether extract and weigh as vanillin, omitting the treatment with ammonia and hydrochloric acid—that is, the procedure which has for its object the separation of vanillin from coumarin.

Note.—For extracting the coumarin and vanillin use ether washed with water, at least twice, to remove alcohol. Dry the extracted coumarin and vanillin over sulphuric acid as directed in the published method, weigh, and then vaporize by heating in a drying oven at 105° C. to constant weight. Heating one hour is usually sufficient. Record the loss of weight as the weight of vanillin or coumarin.

(c) *Normal (neutral) lead number.*—Follow the method given in Bureau of Chemistry Bulletin 137, pages 68 and 120.

(d) *Residual color in filtrate after precipitation with lead acetate.*—Follow the method given in Bureau of Chemistry Circular 90, pages 12 and 13. Also gauge the color value of the filtrate in a 1-inch cell with the "brewer's scale" of Lovibond tint glasses (series 52).

3. *Determination of the color value of the extract.*

(a) Follow the method given in Circular 90, page 12.

(b) Proceed as under (a), except gauge the color with brewer's scale glasses instead of combinations of red and yellow glasses.

(c) Repeat (a) and (b), except dilute 5 cc of the extract to 50 cc and multiply the observed color values by 10 instead of 25.

4. *Per cent of color insoluble in amyl alcohol (Marsh reagent).*

Follow the method given in Bulletin 132, page 90, using 25 cc of the extract, and evaporating only enough to remove the greater part of the alcohol present. Filter the solutions before making the color comparisons. Use any convenient form of colorimeter.

RESULTS.

Reports were received from the following chemists:

1. E. H. Berry, Chicago, Ill.
2. Eugene Bloomberg, Galveston, Tex.
3. C. O. Dodge, Washington, D. C.
4. H. W. Haynes, Detroit, Mich.
5. R. S. Hiltner, Denver, Colo.
6. W. W. Leach, Denver, Colo.
7. C. I. Lott, Buffalo, N. Y.
8. H. J. Wichmann, Denver, Colo.
9. H. E. Woodward, Philadelphia, Pa.

To simplify the presentation of the data in tabular form, the analysts will be referred to by number as above indicated rather than by name.

In Table 1 are given the more significant data obtained in the analyses of the vanilla extract samples:

TABLE 1.—*Results of analyses of vanilla extracts.*

Analyst and sample number.	Wichmann's test for coumarin.	Coumarin.		Vanillin.		Normal lead number.	Residual color in filtrate (provisional method).				Color insoluble in Marsh's reagent.
		Provisional method.	Volatilized.	Provisional method.	Volatilized.		Red.	Yellow.	Total color, red and yellow.	Total color, Brewer's scale.	
Sample 1.		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
Analyst 1	Positive	0.016	0.012	0.160	0.150	0.50	7.8	11.9	10.7	10.3	47.0
2	do	.020	.020	.160	.150	.47				9.8	
3	do	.070	.040	.640	.080	.48	13.3	23.2	20.4	16.0	45.0
4	do	.014	.004	.116	.108					9.5	35.0
5	do					.503	4.8	12.9	10.5	11.2	45.8
6	do										
7	Negative	.017	.014	.166	.158	.52					43.5
8	Positive	.018	.010	.164	.138	.501	8.8	12.8	11.7	10.2	50.3
9	Negative	.018	.014	.177	.153	.59				10.4	46.0
Sample 2.											
Analyst 1	Negative	None.	None.	.200	.190	.50	11.1	12.8	12.3	12.5	23.8
2	do	None.	None.	.220	.210	.39					
3	Positive	0.080	0.060	.230	.120	.23	16.7	20.0	19.0	16.0	26.0
4	Negative	None.	None.	.160	.149					18.0	
5	do	None.	None.	.211	.198	.501	13.3	15.2	14.7	14.0	28.5
6	do	None.	None.	.220	.180	.45	8.48	9.4	9.1	10.6	25.9
7	do	0.006	0.004	.198	.187	.42					27.9
8	do	None.	None.	.206	.197	.455	13.6	14.2	14.1	16.4	27.1
9	do	None.	None.	.227	.206	.56				14.0	25.0
Sample 3.											
Analyst 1	Negative	None.	None.	.340	.320	.55	7.2	8.0	7.8	8.0	22.8
2	do	None.	None.	.340	.300	.45					
3	Positive			.330	.110	.54	5.2	9.6	7.8	6.7	27.0
4	Negative	None.	None.	.300	.290					8.0	20.0
5	do	None.	None.	.356	.331	.615	4.5	7.7	6.9	8.3	24.8
6	do	None.	None.	.350	.320	.62	6.5	8.2	7.9	9.7	22.2
7	do	0.007	0.003	.335	.322	.56					21.3
8	do	None.	None.	.355	.334	.584	6.4	7.7	7.4	8.0	24.9
9	do	None.	None.	.335	.304	.40				8.0	24.0
Sample 4.											
Analyst 1	Negative	None.	None.	.260	.240	.63	7.2	8.0	7.8	8.0	25.0
2	do	None.	None.	.230	.210	.59				7.6	
3	Positive	0.080	0.050	.280	.150	.60	5.4	8.4	7.7	8.0	28.0
4	Negative	None.	None.	.200	.190					7.3	19.0
5	do	None.	None.	.270	.250	.69	5.2	8.0	7.3	8.3	22.5
6	do	None.	None.	.270	.240	.76	6.3	7.7	7.4	8.8	20.3
7	do	0.008	0.004	.250	.240	.64					22.1
8	do	None.	None.	.270	.250	.64	6.7	7.7	7.5	8.0	21.7
9	do	None.	None.	.260	.230	.48				8.0	24.0
Sample 5.											
Analyst 1	Negative	None.	None.	.190	.170	.50	6.3	6.7	6.6	6.8	13.3
2	do	None.	None.	.170	.160	.47				6.4	
3	do			.350	.100	.51	2.3	5.5	4.6	8.0	8.0
4	do	None.	None.	.130	.100	.44				5.7	19.0
5	do	None.	None.	.210	.190	.56	3.1	6.3	5.4	6.3	8.3
6	do	None.	None.	.200	.160	.56	3.8	7.2	7.2	7.2	9.3
7	do	0.026	0.023	.150	.140	.55					7.6
8	do	None.	None.	.200	.170	.53	3.1	6.0	5.3	6.0	8.7
9	do	0.020	0.010	.160	.150	.52				7.0	11.0

TABLE 1.—*Results of analyses of vanilla extracts*—Continued.

Analyst and sample number.	Wichmann's test for coumarin.	Coumarin.		Vanillin.		Normal lead number.	Residual color in filtrate (provisional method).				Color insoluble in Marsh's reagent.
		Provisional method.	Volatilized.	Provisional method.	Volatilized.		Red.	Yellow.	Total color, red and yellow.	Total color, brewer's scale.	
Sample 6.		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
Analyst 1	Negative	None.	None.	.200	.180	.65	6.9	8.1	7.8	7.4	23.8
2	do	None.	None.	.200	.180	.62	.....	.....	.....	7.4	.....
3	Positive	0.080	0.030	.210	.080	.64	2.6	9.4	7.8	8.0	28.0
4	Negative	None.	None.	.170	.160	.63	.....	.....	.....	8.0	24.0
5	do	None.	None.	.210	.190	.72	4.8	8.0	7.1	8.0	21.4
6	do	None.	None.	.130	.150	.75	5.7	7.0	6.8	8.0	22.3
7	do	0.008	0.004	.190	.180	.72	.....	.....	.....	.....	22.6
8	do	None.	None.	.210	.190	.68	4.1	8.2	7.0	7.4	23.6
9	do	None.	None.	.210	.170	.70	.....	.....	.....	8.0	23.0
Sample 7.											
Analyst 1	Negative	None.	None.	.180	.160	.45	5.2	5.9	5.7	6.4	17.4
2	do	None.	None.	.190	.....	.44	.....	.....	.....	6.4	.....
3	Doubtful	0.060	0.030	.170	.080	.44	1.9	5.9	5.0	8.0	20.0
4	Negative	None.	None.	.120	.100	.45	.....	.....	.....	4.8	20.0
5	do	None.	None.	.190	.170	.48	3.8	6.4	5.6	7.1	17.7
6	do	None.	None.	.170	.150	.47	4.1	6.0	5.6	7.0	14.1
7	do	0.015	0.013	.160	.150	.47	.....	.....	.....	.....	16.6
8	do	None.	None.	.190	.170	.45	3.5	5.9	5.3	6.4	15.5
9	do	None.	None.	.170	.150	.44	.....	.....	.....	7.2	20.0

The results of the study of the color values of vanilla extracts are given in the following table:

TABLE 2.—*Color value of vanilla extracts.*

Analyst and sample number.	Color value of extract, 1-inch cell Lovibond color scale.								Color value of filtrate, 1-inch cell Lovibond color scale.				Residual color in filtrate calculated from results.								Ratio of red to yellow color.		
	Provisional method: factor×25.				Method 3 (c): factor×10.								Method 3 (a).				Method 3 (c).				In extract.		
	Red.	Yellow.	Total color, red and yellow.	Total color by brewer's scale.	Red.	Yellow.	Total color, red and yellow.	Total color by brewer's scale.	Red.	Yellow.	Total color, red and yellow.	Total color by brewer's scale.	Red.	Yellow.	Total color, red and yellow.[1]	Total color by brewer's scale.	Red.	Yellow.	Total color, red and yellow.[1]	Total color by brewer's scale.	Factor 25.	Factor 10.	In filtrate.
Sample 1.													*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*			
Analyst 1	51.0	123.0	174.0	213.0	42.0	150.0	192.0	265	4.0	14.6	18.6	22.0	7.8	11.9	10.7	10.3	9.5	9.7	9.6	8.3	1:2.4	1:3.6	1:3.7
2				225.0				260				22.0				9.8				8.5			
3	48.7	122.5	171.2	225.0	50.1	211.0	261.1	270	6.5	28.4	34.9	36.0	13.3	23.2	[2]20.4	[2]16.0	13.0	17.2	[2]16.5	[2]14.4	1:2.5	1:4.2	1:4.4
4				200.0				230				19.0				9.5				8.3			
5	47.5	125.0	172.5	187.5	46.0	145.0	191.0	249	2.2	16.2	18.2	21.0	4.8	12.9	10.5	11.2	14.8	11.1	9.5	8.4	1:2.6	1:3.1	1:7.3
6																							
7				200.0				245				20.0											
8	50.0	140.0	190.0	225.0	50.0	170.0	220.0	230	4.4	18.0	22.4	23.0	8.8	12.8	11.7	10.2	8.8	10.6	10.1	10.0	1:2.8	1:3.4	1:4.0
9				212.5				240				22.0				10.4				9.2			
Average															10.9	10.2			9.7	8.8			
Sample 2.																							
Analyst 1	18.0	50.0	68.0	88.0	18.0	54.0	72.0	80	2.0	6.4	8.4	11.0	11.1	12.8	12.3	12.5	11.1	11.8	11.6	13.7	1:2.8	1:3.0	1:3.2
2				100.0				95				0.0											
3	21.0	50.0	71.0	100.0	18.5	58.3	76.8	90	3.5	10.0	13.5	16.0	16.7	20.0	19.0	16.0	18.9	17.1	17.6	17.7	1:2.4	1:3.1	1:2.9
4				100.0				90				18.0				18.0				20.0			
5	19.5	52.5	72.0	92.5	18.0	54.0	72.0	85	2.6	8.0	10.6	13.0	13.3	15.2	14.7	14.0	14.4	14.8	14.7	15.3	1:2.7	1:3.0	1:3.1
6	16.5	48.8	65.3	75.0	18.0	58.0	76.0	80	1.4	4.6	6.0	8.0	8.5	9.4	9.1	10.6	7.7	7.8	7.9	10.0	1:2.9	1:3.2	1:3.2
7				87.5				90															
8	18.5	46.3	64.8	87.5	19.0	56.0	75.0	85	2.6	8.0	10.6	14.0	13.6	14.2	14.1	16.4	14.0	17.2	16.3	16.0	1:2.5	1:2.9	1:3.3
9				100.0				90				14.0				14.0				15.5			
Average															13.8	14.5			13.6	15.4			
Sample 3.																							
Analyst 1	25.0	75.0	100.0	138.0	26.0	84.0	110.0	130	1.8	6.0	7.8	11.0	7.2	8.0	7.8	8.0	6.9	7.1	7.1	8.5	1:3.0	1:3.2	1:3.3
2												10.0											
3	25.0	78.9	103.9	150.0	23.0	89.0	112.0	125	1.3	6.8	8.1	10.0	5.2	9.6	7.8	6.7	5.6	7.6	7.2	8.0	1:3.1	1:3.8	1:5.2
4				125.0				120				10.0				8.0				8.3			
5	25.0	75.0	100.0	120.0	26.0	92.0	118.0	115	1.1	5.8	6.9	10.0	4.5	7.7	6.9	8.3	4.3	6.3	5.8	8.7	1:3.0	1:3.5	1:5.2
6	22.5	77.5	100.0	112.5	21.0	88.0	109.0	110	1.5	6.4	7.9	11.0	6.5	8.2	7.9	9.7	7.0	7.2	7.2	10.0	1:3.4	1:4.2	1:4.3
7				137.5				125															
8	25.0	75.0	100.0	125.0	25.0	90.0	115.0	125	1.6	5.8	7.4	10.0	6.4	7.7	7.4	8.0	6.4	6.4	6.4	8.0	1:3.0	1:3.6	1:3.6

9				137.5				130				11.0				8.0				8.5			
Average															7.4	8.1			6.7	8.5			
Sample 4.																							
A yst 1	25.0	75.0	100.0	138.0	26.0	84.0	110.0	130	1.8	6.0	7.8	11.0	7.2	8.0	7.8	8.0	6.9	7.1	7.1	8.5	1:3.0	1:3.2	1:3.3
2				131.0				125				10.0				7.6				8.0			
3	23.1	76.4	99.5	125.0	22.0	90.0	112.0	120	1.3	6.4	7.7	10.0	5.4	8.4	7.7	8.0	5.7	7.1	6.9	8.3	1:3.3	1:4.1	1:5.1
4				137.5				130				10.0				7.3				7.7			
5	23.8	75.0	98.8	137.5	23.0	94.0	117.0	120	1.2	6.0	7.2	11.4	5.2	8.0	7.3	8.3	5.3	6.4	6.2	9.5	1:3.1	1:4.1	1:4.8
6	19.5	75.0	94.5	112.5	19.5	94.0	113.5	110	1.2	5.8	7.0	10.0	6.3	7.7	7.4	8.8	6.3	6.4	6.2	9.9	1:3.8	1:4.8	1:4.6
7				137.5				130															
8	23.8	77.5	101.3	125.0	22.0	94.0	116.0	120	1.6	6.0	7.6	10.0	6.7	7.7	7.5	8.0	7.2	6.3	6.5	8.3	1:3.2	1:4.2	1:3.7
9				137.5				130				11.0				8.0				8.5			
Average															7.5	8.0			6.6	8.6			
Sample 5.																							
Analyst 1	16.0	48.0	64.0	88.0	17.0	51.0	68.0	80	1.0	3.2	4.2	6.0	6.3	6.7	6.6	6.8	5.9	6.3	6.2	7.5	1:3.0	1:3.0	1:3.2
2				93.0				90				6.0				6.4				6.7			
3	20.0	48.7	68.7	75.0	17.5	52.0	69.5	80	.5	2.7	3.2	6.0	2.3	5.5	4.6	8.0		5.2	4.5	7.5	1:2.4	1:3.0	1:5.9
4				87.5				85				5.0				5.7				5.9			
5	19.0	48.8	67.8	95.0	17.5	55.0	72.5	85	.6	3.1	3.7	6.0	3.1	6.3	5.4	6.3	3.4	5.6	5.1	7.0	1:2.6	1:3.1	1:5.0
6	20.5	50.0	70.5	82.5	16.5	64.0	80.5	80	.8	3.6	4.4	6.0	3.8	7.2	7.2	7.2	4.8	5.6	5.4	7.5	1:2.4	1:3.8	1:3.5
7				87.5				85															
8	15.5	50.0	65.5	100.0	19.0	55.0	74.0	85	.5	3.0	3.5	6.0	3.1	6.0	5.3	6.0	2.5	5.4	4.7	7.0	1:3.2	1:2.9	1:6.2
9				100.4				90				7.0				7.0				7.8			
Average															5.8	6.6			5.2	7.1			
Sample 6.																							
Analyst 1	35.0	108.0	143.0	175.0	32.0	127.0	159.0	180	2.4	8.8	11.2	13.0	6.9	8.1	7.8	7.4	7.5	6.9	7.0	7.2	1:3.1	1:3.9	1:3.7
2				175.0				190				13.0				7.4				6.8			
3	34.4	106.0	140.4	175.0	35.0	142.5	177.5	180	.9	10.0	10.9	14.0	2.6	9.4	7.8	8.0	2.6	7.0	6.1	7.8	1:3.0	1:4.0	1:11.0
4				162.5				145				13.0				8.0				9.0			
5	37.5	100.0	137.5	162.5	39.0	125.0	164.0	170	1.8	8.0	9.8	13.0	4.8	8.0	7.1	8.0	4.1	6.4	6.0	7.7	1:2.7	1:3.2	1:4.4
6	31.3	125.0	156.3	162.5	35.0	130.0	165.0	160	1.8	8.8	10.6	13.0	5.7	7.0	6.8	8.0	5.1	6.7	6.4	8.1	1:4.0	1:3.7	1:4.8
7				175.0				155															
8	40.0	100.0	140.0	175.0	40.0	132.0	172.0	180	1.7	8.2	9.9	13.0	4.1	8.2	7.0	7.4	4.1	6.2	5.7	7.2	1:2.5	1:3.3	1:4.9
9				187.5				180				15.0				8.0				8.3			
Average															7.3	7.8			6.2	7.7			
Sample 7.																							
Analyst 1	23.0	68.0	91.0	125.0	21.0	71.0	92.0	110	1.2	4.0	5.2	8.0	5.2	5.9	5.7	6.4	5.7	5.6	5.7	7.2	1:3.0	1:3.4	1:3.3
2				125.0				125				8.0				6.4				6.4			
3	21.0	71.2	92.2	100.0	21.0	80.0	101.0	105	.4	4.2	4.6	8.0	1.9	5.9	5.0	8.0	1.9	5.3	4.5	7.6	1:3.3	1:3.8	1:10.5
4				125.0				110				6.0				4.8				5.5			
5	27.5	65.0	92.5	117.5	24.0	86.0	110.0	115	1.1	4.2	5.3	8.4	3.8	6.4	5.6	7.1	4.3	4.9	4.7	7.3	1:2.3	1:3.6	1:4.0
6	16.5	65.0	81.5	100.0	19.0	84.0	103.0	100	.7	3.9	4.6	7.0	4.1	6.0	5.6	7.0	3.5	4.6	4.4	7.0	1:3.9	1:4.4	1:5.7
7				125.0				115															
8	22.5	67.5	90.0	125.0	23.0	87.0	110.0	115	.8	4.0	4.8	8.0	3.5	5.9	5.3	6.4	3.4	4.6	4.3	6.9	1:3.0	1:3.7	1:5.0
9				125.0				120				9.0				7.2				7.5			
Average															5.4	6.6			4.7	6.9			

[1] Calculated by the formula: (Red+yellow in filtrate)÷(Red+yellow in extract)×100.

[2] Omitted from average.

Reference to the data in the tables shows, among other facts, that Wichmann's test for coumarin did not give uniformly correct results; only one sample contained coumarin, namely, No. 1. In the opinion of the associate referee this method may be relied upon for the detection of small amounts of coumarin, and answers well the purpose intended. The few failures to report correctly on the presence of coumarin in the samples sent out this year probably resulted from the fact that the details of procedure were not stated clearly enough, rather than from any inherent fault in the chemistry of the operation. The principal difficulty seems to be in controlling properly the temperature when heating the distillate with potassium hydroxid to convert the coumarin into potassium salicylate. After evaporating to dryness the residue should be heated cautiously only to the point of incipient fusion.

Results received from the collaborators for percentage of coumarin, vanillin, total color, and color insoluble in Marsh's reagent, and for lead number were in the main quite satisfactory. It is evident that the percentages of vanillin and coumarin as found by the provisional method are invariably somewhat too high, as pointed out in the report of last year. Probably the proposed method of obtaining results by volatilization gives more nearly correct figures. The difference, however, from those given by the provisional method is in every case small, seldom more than 0.02 per cent, so that it seems inadvisable to recommend the adoption of the proposed method, especially since it offers no feasible means of determining the purity of the ether extract.

The principal objection that can be raised to the provisional method of determining the percentages of the component colors red and yellow remaining in the filtrate after precipitation with lead acetate is that few laboratories have at hand the necessary equipment. The results obtained by the method are consistent in every case, and it would be well, therefore, for the association to retain this as a provisional method. While it is true in many cases that the series of Lovibond color glasses, known as the brewer's scale, does not always give combinations that are strictly comparable with the colors found in vanilla extracts and in the filtrates after treatment with lead acetate, it is evident from the results reported that very significant data may be obtained, and the figures showing percentages compare very favorably with the percentages of total red and yellow, as found by the Winton-Berry method. Nearly all food-inspection laboratories now have in stock a set of brewer's scale color glasses, and hence the method may be used to advantage instead of the more elaborate procedure of estimating the percentage of component colors.

Apparently one of the surest methods of detecting caramel in vanilla extracts is by determining the percentage of color insoluble in acidified amyl alcohol (Marsh's reagent). It may be seen from Table 1, in the data for Sample 1, that the percentages of residual color in the filtrate fail to indicate the presence of artificial color. There is more residual color in the filtrate of Sample 2 than in Sample 1. Sample 1 contains added caramel, whereas I was positively assured by the manufacturer of Sample 2 that no caramel or other artificial color was used. The percentage of color insoluble in Marsh's reagent in Sample 1 is very much larger than in Sample 2 and is far above the normal figure for pure vanilla extract.

No advantage seems to be gained in determining the color value of extracts by using a 10 per cent solution instead of a 4 per cent, as required by the provisional method, except when the color is gauged with the brewer's scale glasses. The use of the smaller factor 10 in this instance gives generally more uniform results.

There was a diversity of opinion among the collaborators as to whether results of analysis should be computed as grams per 100 cc or as per cent by weight. Six analysts were in favor of reporting results as grams per 100 cc and three held that the provisional method requiring percentages by weight was to be preferred. On this subject Mr. Bloomberg said: "I am in favor of having results expressed as per cent by weight, owing to the awkwardness of having to keep the extracts at the low temperature of 20° C. when measurements are made. This is more especially the case in this laboratory, where the average temperature for six or eight months of the year is 30° C. or above."

The associate referee still holds to the opinion that it is better to express results as grams per 100 cc, for the reasons given in the report last year.[1] He requests that this matter be considered by the association at its next meeting and decided by vote of the section.

CONCLUSIONS.

As to the limits of composition of pure standard vanilla extracts, when examined by the provisional method, the results received from the nine analysts seem to warrant the following conclusions:

First, that the percentage of vanillin should be not less than 0.10 nor more than 0.35.

Second, that the normal or neutral lead number should be not less than 0.40 nor more than 0.80.

Third, that the percentage of total color remaining in the filtrate after precipitation with lead acetate should be not more than 15.

Fourth, that the percentage of color insoluble in acidified amyl alcohol (Marsh's reagent) should be not more than 35, and will seldom exceed 25 per cent.

These conclusions are in harmony with those obtained by Winton and Berry and are in line also with the data reported last year from standard laboratory-made extracts.

Ginger Extracts.

Only one sample of ginger extract was submitted. The tincture was made according to the United States Pharmacopœia formula from a mixture of Japan and Cochin ginger. It was assumed that the analyses of this one sample would suffice to show the value of the proposed methods. The details of procedure were essentially the same as proposed by Street and Morrison [2] and were as follows:

METHODS.

1. *Alcohol by volume.*—Pipette 25 cc of the sample into a 100 cc flask and dilute to the mark with water. Shake with 2 or 3 grams of dry magnesium carbonate and filter. Distill 75 cc of the filtrate into a 50 cc flask. Ascertain the per cent alcohol by volume in the distillate from its specific gravity and multiply by 8/3.

2. *Total solids* (grams per 100 cc).—(a) Follow the method in Bulletin 137, page 79, except the use of 10 cc of the sample instead of 5 to 10 grams.

(b) Pipette 10 cc of the extract into a tared flat-bottomed dish (about 4 inches in diameter). Dry to constant weight at room temperature in a desiccator over sulphuric acid.

3. *Alcohol-soluble solids* (grams per 100 cc).—Follow the method in Bulletin 137, page 79, using the residue obtained under 1 (a).

4. *Water-soluble solids* (grams per 100 cc).—Follow the method in Bulletin 137, page 79, using the residue obtained under 1 (b).

5. Compare the sensitiveness of Seeker's and Mitchell's tests for ginger as follows: Mix 5 cc of the extract with 5 cc of glycerin, 10 cc of water, 5 drops of caramel, and complete the volume to 100 cc with 95 per cent alcohol. Then test 10 cc of the diluted sample by Seeker's method as directed in Bulletin 137, page 75, and by Mitchell's method as given in Circular 90, page 13.

RESULTS.

Five analysts reported results which are recorded in the following table:

Analyst.	Alcohol by volume.	Total solids, Method 2 (a).	Total solids, Method 2 (b).	Alcohol-soluble solids.	Water-soluble solids.
	Per cent.	*Grams per 100 cc.*	*Grams per 100 cc.*	*Grams per 100 cc.*	*Grams per 100 cc.*
Eugene Bloomberg, Galveston, Tex	84.53	1.38	1.56	1.344	0.396
C. O. Dodge, Washington, D. C	90.80	1.33	1.54	1.24	.40
H. B. Gordon, Chicago, Ill	89.50	1.374	1.635	1.308	.342
H. W. Haynes, Detroit, Mich	85.51	1.316	1.581	.66	.175
R. S. Hiltner, Denver, Colo	93.4	1.37	1.54	1.30	.43
C. I. Lott, Buffalo, N. Y	92.08	1.324	1.578	.310	.294

[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 152, p. 135.
[2] U. S. Dept. Agr., Bureau of Chemistry Bul. 137, p. 79.

It is apparent that the method for the determination of alcohol-soluble and water soluble solids requires further investigation, particularly the former. These two determinations are the most significant of the group; hence the method involved should be perfected so as to yield concordant results.

The trend of opinion expressed by the collaborators as to the relative merits of the Seeker and Mitchell tests for ginger was that both could be relied upon to give correct results, and that Mitchell's method gave a stronger color and was quicker than Seeker's. It is suggested, therefore, that the Mitchell modification be approved as an optional method.

Recommendations.

It is recommended—

(1) That the limits of composition of standard vanilla extracts, as above stated, be accepted tentatively by the association and published in its Proceedings.

(2) That the study of the composition of pure commercial vanilla extracts be continued next year for the purpose of securing additional data.

(3) That Wichmann's method for detecting coumarin be further studied.

(4) That the study of the methods for the examination of ginger extracts and other flavoring materials, as recommended last year, be made the subject of further work next year.

THE COMPOSITION OF VANILLA EXTRACT FROM TAHITI AND FIJI BEANS.

By A. L. Winton and E. H. Berry.[1]

In the Proceedings of the association for 1911 are given analyses by the writers of vanilla extracts from different varieties, grades, and lengths of vanilla beans, also of extracts prepared by different processes. From these analyses it appears that the extract of Tahiti beans, a variety of recognized inferiority, is characterized by its low vanillin content and low color value. These deficiencies are due in part to the high moisture content of these beans, because of which some manufacturers dry the beans before making the extract.

The law requiring the inspection of vanilla beans before exportation, which went into effect in French Oceania in 1911, is stated to have resulted in a marked improvement in the quality of the bean from Tahiti. For the purpose of extending our knowledge of the composition of vanilla extracts prepared from Tahiti beans, particularly those exported under the new system of inspection, extracts were prepared by the United States Pharmacopœia process from three grades obtained from a well-known importer. Extracts were also prepared from a Fiji bean and from the residues of the United States Pharmacopœia process by long soaking in 60 per cent alcohol, as described in last year's report. Analyses of all of these samples appear in the following table, the results in the case of the extracts from residues, although representing 20 grams of beans per 100 cc, being calculated to the 10-gram basis for comparison:

[1] In the absence of the authors, the paper was presented by W. D. Bigelow.

Analyses of United States Pharmacopœia vanilla extracts made from Tahiti and Fiji beans and from the residues after extraction.

Kind of bean.	Length of bean.	Vanillin.	Normal lead number.	Color value.				Total color in lead filtrate.		Ratio of red to yellow.		Total color insoluble in amyl alcohol.
				Extract (total color).		Lead filtrate.				Extract.	Lead filtrate.	
				Red.	Yellow.	Red.	Yellow.	Red.	Yellow.			
U. S. P. extracts:												
Tahiti—	*Cm.*	*P. ct.*	*P. ct.*					*P. ct.*	*P. ct.*			*P. ct.*
Dry beans[1].	15	0.17	0.56	13	38	0.8	3.0	6	8	1:2.9	1:3.7	18
Wet beans[2].	16	.12	.50	13	39	.8	3.4	6	9	1:3.0	1:4.2	22
Transplanted Mexicans[3]	17	.17	.53	15	46	.8	3.5	5	8	1:3.0	1:4.3	19
Fiji[4].....	18	.15	.68	28	88	1.8	7.1	6	8	1:3.1	1:3.9	29
Extracts from residues:												
Tahiti—												
Dry beans.....		.03	.09	11	32	.4	1.2	4	4	1:2.9	1:3.0	11
Wet beans.....		.03	.06	9	28	.3	.9	3	3	1:3.1	1:3.0	10
Transplanted Mexicans.....		.03	.09	12	36	.4	1.2	3	3	1:3.0	1:3.0	10
Fiji.....		.04	.10	13	40	.6	1.8	5	5	1:3.1	1:3.0	13

[1] Moisture, 45.52 per cent.
[2] Moisture, 52.26 per cent.
[3] Moisture, 38.16 per cent.
[4] Moisture, 17.14 per cent.

From these results it appears that the Tahiti extracts, like those examined last year, are of an abnormally light color, but that the Fiji extract is quite as dark as that made from many samples of other grades of beans.

The vanillin content of the so-called wet Tahiti beans is low, but that of the dried beans and the transplanted Mexicans is nearly as high as good grade extracts made from Mexican and Bourbon beans. Taking into account the low moisture content of the Fiji beans, the vanillin content is no higher than that of the wet Tahiti beans.

A paper by Charles F. Poe, of the University of Colorado, on "A probable method for the estimation of optically active oils in extracts," was read by W. D. Bigelow and will be published in some chemical journal after more work has been done by the author.

The following paper by Mr. Hiltner was presented by A. S. Mitchell:

A METHOD FOR THE DETECTION OF CARAMEL IN TINCTURES AND EXTRACTS OF GINGER.

By R. S. Hiltner.

The coloring matter of pure ginger extracts is completely soluble in acidified amyl alcohol (Marsh's reagent) while caramel is insoluble. On the basis of these facts the following qualitative and approximately quantitative method is proposed:

Proceed as directed in Bureau of Chemistry Bulletin 132, page 90 (Tolman's method for color in distilled liquors), and note the resulting colors in the two separated layers of liquid. In case the lower (aqueous) layer is colorless, the absence of caramel or similar color is indicated. If caramel be present, the lower layer will be found to be colored yellowish-brown, the intensity of color being proportionate to the amount of foreign color present. By following Tolman's method of comparing the color value of the original sample with that of the portion extracted with the Marsh reagent, the approximate amount of added color may be determined.

REPORT ON SPICES.

By R. W. Hilts, *Associate Referee.*[1]

This year's work has been limited to a continuation of the study of the ether extract of paprika, particularly of the refractive index with reference to the detection of added oils. Collaborative samples were not sent out, as the investigation seemed to be still in a preliminary stage. Samples of the whole fruit and also of a number of commercial ground lots were examined. Three samples of the whole pods were available, one Hungarian and two Spanish, Sample A being the same lot as used in last year's work. The shells and seeds were ground separately, after rejecting the stems and placentæ. Sample A, Spanish pimenton, then contained 63.9 per cent of shells and 36.1 per cent of seeds. The normal proportion could not be determined in the other two samples, as the pods were slit open and many of the seeds lost.

For the refractive index about 8 grams of sample were digested for one or two hours with about 40 cc of pure anhydrous ether. This was thrown on a dry filter, the ether evaporated, and the extract dried for one-half hour in the water oven. The Abbé refractometer (Zeiss) was used at a temperature of 25° C. Much difficulty was found in reading the oil from the shells and from the whole pods, owing to its intense red color, which hides the play of color on the border line due to dispersion, and also on account of the poor definition of the line. If this trouble were due to some constituent near the temperature at which it would separate, higher temperature should correct this, but the reading was no better at 40° C. than at 15° C. As the great difficulty was in fixing the position of the compensator, sodium light was tried to dispense with the necessity for compensation. This was not successful, probably because of poorer illumination. By repeated trials and comparison with the sodium flame, however, it was found that compensation and definition were best with the compensator set at about 31.5, using daylight, and with the tube of the instrument set nearly vertical. All readings on the red oils were accordingly made under these somewhat arbitrary conditions. The yellow oil from the seeds offered no difficulties. The ether extract and iodin number of the oils were by the provisional methods.

Determinations on the whole pods.

Sample.	Ether extract.		Iodin number.		Index of refraction, 25° C.	
	Shells.	Seeds.	Shells.	Seeds.	Shells.	Seeds.
	Per cent.	*Per cent.*				
(A) Spanish	3.99	18.22	151.2	133.3	1.5262	[1] 1.4741
(B) Spanish	4.44	16.15	142.5	135.3	1.5127	1.4740
(C) Hungarian	5.43	19.29	126.2	132.0	1.4981	1.4737

[1] Oil from shells and seeds ground together gave index of 1.4888.

The indices of refraction of both seeds and shells are well above those of the common oils, though a few of the drying oils would approach the seed oil. The variations noted on the shells, however, are so great that they destroy the value of the index for detecting foreign oils. Moreover, because of the difference between shells and seed, the index of the ether extract from the whole ground pod will be influenced by the proportion of shells and seeds in a given sample, which introduces another uncertain factor and further decreases the value of the index for detecting foreign oils. It was hoped, however, that even if the index were useless for this purpose, it might still, when taken in conjunction with other data, have value in proving the presence of an

[1] Read by H. E. Sindall.

added excess of seeds, a form of adulteration undoubtedly practiced in grinding low-grade paprikas. In the above data it is of interest to note that in two cases the oil from the shells had a distinctly higher iodin number than that from the seeds, which appears to be unusual.

The index of refraction of the oil was then determined on a number of commercial samples, taken from direct importations and of known geographical origin. The data are tabulated in order of increasing index. A rough grading as to color is given, since this might be influenced by excess of seeds.

Determinations on commercial peppers.

Sample number.	Source.	Color.	Ether extract.	Iodin number.	Index of refraction at 25° C.
			Per cent.		
1	Spain	Very poor.	14.78	122.1	1.4754
2	do	Poor	14.08	118.7	1.4757
3	do	do	12.71	124.5	1.4766
4	Hungary	do	13.80	137.0	1.4800
5	do	do	12.76	135.2	1.4802
6	Spain	Good	11.42	124.2	1.4817
7	Hungary	do	12.58	131.3	1.4824
8	Spain	do	11.85	124.8	1.4824
9	do	do	11.02	128.9	1.4830
10	Hungary	do	13.16	137.3	1.4836
11	Spain	do	10.91	128.6	1.4838
12	do	do	11.30	126.4	1.4842
13	Hungary	do	15.04	136.1	1.4846
14	Spain	do	11.69	130.9	1.4857
15	Hungary	do	14.44	137.4	1.4887

If the index of refraction is influenced by the relative proportion of shells and seeds in a given sample, as would appear from the examination of the known samples, high indices should accompany low ether extracts and vice versa, if foreign oils are absent. Unfortunately no such relation can be seen in the above data. The first two samples may be excluded from this consideration, as they probably contain a little added oil. The Spanish and Hungarian peppers are not distinguished by the indices of the oil. The referee is compelled to conclude that the index of refraction of the ether extract has no value in detecting added foreign oil, nor in detecting excess of seeds, because of the large variations in individual samples. It might have some confirmative value in the case of gross adulteration with oil.

During the past year v. Sigmond and Vuk[1] have published work on the detection of added oil in paprika. They reject the refractive index as useless and further state that as little as 1 to 2 per cent of oil, as rape, is often added in grinding. Our present methods could not detect this small addition with certainty. These authors suggest some mechanical tests, as determining the oil yielded by the sample to paper under pressure, that might be successfully worked out, though abandoned by these investigators.

Thanks are due to A. F. Seeker for samples of whole paprika, and to C. S. Brinton and H. E. Woodward for some of the data on commercial samples.

Recommendations.

It is recommended—

(1) That an effort be made to devise methods other than microscopical for detecting an excess of seeds in paprika.

(2) That, if possible, samples of prepared mustard of known composition be submitted to collaborators for the determination of crude fiber by the present official methods.

[1] Zts. Nahr. Genussm. 1911, *22:* 599; 1912, *23:* 387.

THE CHLORAL HYDRATE TEST FOR CHARLOCK.

By A. L. Winton.[1]

Wagge[2] appears to have originated this test. At least he applied it to the detection of charlock in mustard flour nearly 20 years ago. It consists in mounting a portion of the material on a microscopic slide in chloral hydrate solution (8:5) and noting the Carmine Red color formed in and about fragments of the hulls of charlock. The reaction takes place slowly in the cold but almost immediately on gentle heating. None of the true mustards give this color,

Under the compound microscope it may be seen that the color is due to the partial solution of the dark colored contents of the palisade cells which form the third layer of the hull. Even without this color reaction, identification of charlock is not difficult for the microscopist, as none of the true mustards have this dark substance in the palisade cells; furthermore, as shown by Collins,[3] the epidermal cells, especially when viewed with polarized light, are characteristic. The compound microscope, however, is not essential, or even desirable, for the color test, since, as proposed by K. B. Winton, a better idea of the proportion of charlock is gained by examination with a strong lens, holding the mount in front of a piece of white paper before a strong light.

For years the writer has applied this test successfully until recently, when a new lot of chloral hydrate solution was found to give only a faint reaction, so faint, indeed, as to be indecisive. Thinking that the reagent was impure, other lots were tried with the same result, while a solution made up several months before invariably gave sharp reactions, indicating that the reagent is only active after long standing. In searching for the reason for this difference, it was soon found that the reaction of the fresh solutions was in all cases neutral, whereas that of the old solution was strongly acid. This is in accordance with a statement made in the United States Pharmacopœia.

As the acid formed is doubtless hydrochloric acid, it was reasoned that the addition of this acid to fresh chloral hydrate solution should bring about the same result as aging, and further experiment proved this to be the case. A mixture of 1 volume of concentrated hydrochloric acid with 20 volumes of chloral hydrate solution of the usual strength gave more striking reactions than any obtained with the aged solution.

Further experiments were made to determine whether hydrochloric acid alone or other reagents would produce the same results. It was found that the acid alone gave a faint but unsatisfactory reaction, owing probably to the lack of penetrating power of the acid as compared with chloral hydrate solution, which is a well-known clearing agent, penetrating cell walls and dissolving cell contents. A mixture of 20 volumes of glycerin (another well-known clearing agent) with 1 volume of concentrated hydrochloric acid was found, however, to give a satisfactory color reaction after cautious heating. Sirupy zinc chlorid solution mixed with hydrochloric acid in the same proportion also gave the Carmine Red color on heating, from which it appears that organic matter is not essential for the test.

To learn whether hydrochloric acid is essential, tests were made using sulphuric acid, sirupy phosphoric acid, and a sirupy solution of citric acid. Of these the sulphuric acid was found unsatisfactory because charring set in before reactions could be noted, but phosphoric acid and citric acid gave excellent reactions. It seemed unnecessary to search further, as it was evident that the color could be produced by various acid solutions provided they possessed penetrating power. In all cases heating was essential for a sharp and immediate reaction, acid chloral hydrate solution, however, requiring less heating than any of the other reagents noted.

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[1] Read by W. D. Bigelow in the absence of the author.

[2] Ber. d. Pharm. Ges., 1893, 3:153.

[3] A Note on Mustards and their Chief Adulterant, Charlock. Swarthmore, Pa., 1910.

For general use in detecting charlock the writer prefers the reagent prepared as follows: Dissolve 16 grams of crystallized chloral hydrate in 10 cc of water. To the solution add 1 cc of concentrated hydrochloric acid.

In making the test mount about 10 mg of the mustard flour (or an equivalent amount of prepared mustard) on a slide in the reagent, heat cautiously (never to boiling) for a moment, and examine under a lens. Note the proportion of fragments of hulls that acquire a Carmine-Red color (charlock) to those not changed in color.

REPORT ON BAKING POWDERS.

By Edmund Clark, *Associate Referee.*

The work your associate referee on baking powders hoped to undertake last year was along the lines of adapting his own modification of the Marsh Berzelius method to the determination of arsenic in baking-powder materials, but too large a part of the year was devoted to obtaining arsenic-free reagents and standardizing the method. Particular trouble was found with hydrochloric acid, and only after repeated attempts was this arsenic-free reagent found.

The method under consideration has been published as Circular 99 of the Bureau of Chemistry. Its important features are the very great accuracy and delicacy, brought about by a generator capacity of 8 to 10 cc, and the artificial cooling of the deposition tube at the exact point where it is desired that the mirror be deposited. Although at this time the method is only adapted to products which can be digested to a clear solution with sulphuric acid and nitric acid, it is a matter of only a small amount of work to make the method efficient with the use of hydrochloric acid.

Lead in cream of tartar, as it is introduced into that commodity through the use of sulphuric acid, and the matter of its accurate quantitative estimation present increasingly important questions. No cooperative work was done, but attention is called to electrolytic methods giving good results in the hands of the operators.

The determination of arsenic and lead may properly be taken up by the associate referee on heavy metals, yet the prevalence of arsenic in calcium acid phosphate and of lead in cream of tartar renders them fit subjects for the attention of the associate referee on baking powders. I therefore recommend that the work of investigating the methods of arsenic and lead determinations and adapting them to baking-powder ingredients be continued.

REPORT ON MEAT AND FISH.

By W. B. Smith, *Associate Referee.*

Three determinations were studied this year: Starch, ammonia, and nitrates.

Starch.

The last referee recommended a search for a better method for determining starch in meat products. Since Olsen's method for quick hydrolysis of starch was published attempts have been made to adapt it to meats. No cooperative samples were sent out, because of the perishable nature of the products; but two modifications were tried, by different chemists, with the results reported below. The method suggested by W. H. Low is as follows:

Low method.

Place 2 grams of the ground-up and well-mixed meat in an 800 cc Kjeldahl flask and add 10 cc of water and 6 cc of strong sulphuric acid. Boil the mixture until the meat is practically in solution and fairly clear; two or three minutes will usually suffice. Add 12.5 cc of water and boil; cool and transfer to a 450 cc Erlenmeyer flask; neutralize

with about 18 cc of 36° lye (400 grams of sodium hydroxid to a liter), using phenolphthalein as indicator. The solution will measure 75 to 90 cc. Treat with Fehling's solution and determine the precipitated copper by Low's iodid method. Dextrose times 0.9 equals starch.

The figures obtained by this method are often close enough where great accuracy is not required and where time is a great object.

Results on determination of starch by the Low method.

Cereal added.	Starch found.	Starch found of cereal added.	Analyst.	Cereal found, estimated.	Calculated error.
Per cent.	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*
0.51	0.48	.....	C. T. Allcutt	0.69	+0.18
.52	.28	53.9	.....do	.40	− .12
1.03	.94	.....	.....do	1.34	+ .31
1.02	.59	57.8	.....do	.84	− .18
1.10	.55	50.0	.....do	.80	− .30
2.70	1.53	56.7	.....do	2.19	− .51
5.00	3.54	70.8	.....do	5.06	+ .06
5.02	3.60	71.7	.....do	5.14	+ .12
5.06	3.78	74.7	.....do	5.40	+ .34
5.06	3.15	62.3	.....do	4.50	− .56
5.00	[1] 3.90	[1] 78.0	L. S. Bushnell	[1] 5.59	+ .59
	[2] 3.63	[2] 72.6	.....do	[2] 5.19	+ .19
	[3] 3.78	[3] 75.6	.....do	[3] 5.40	+ .40
1.90	1.25	65.8	W. B. Smith	1.79	− .11
1.90	1.12	59.0	.....do	1.60	− .30
1.90	1.10	57.9	.....do	1.57	− .33
1.90	1.18	62.1	.....do	1.69	− .21
2.00	1.21	60.1	.....do	1.73	− .27
2.00	1.54	77.0	.....do	2.20	+ .20
2.00	1.36	68.0	.....do	1.94	− .06
2.00	1.38	69.0	.....do	1.97	− .03
2.94	1.78	60.3	.....do	2.54	− .40
3.91	2.86	73.1	.....do	4.09	+ .18
4.54	3.46	76.2	.....do	4.94	+ .40
4.86	2.96	60.9	.....do	4.23	− .63
5.00	3.17	63.4	.....do	4.53	− .47
6.32	4.77	75.5	.....do	6.81	+ .49
[4] 3.01	.....	.....	.....	[4] 2.94	.....

[1] Maximum. [2] Minimum. [3] Average. [4] General average.

Assuming arbitrarily that there was 70 per cent of starch in the cereal added, the starch found is calculated to cereal in the fourth column, the results ranging from 0.60 per cent above to 0.60 per cent below on 5 per cent of cereal.

On cereals, chiefly corn flour, such as are used in meat products, the following figures were obtained:

Starch in cereals.

Sample No.	Starch.	Analyst.	Starch.	Analyst.	Sample No.	Starch.	Analyst.	Starch.	Analyst.
	Per cent.		*Per cent.*			*Per cent.*		*Per cent.*	
1.....	61.2	Smith...	71.4	Allcutt.	4.....	64.6	Smith...	.....	
	67.4	....do....	67.4	Do.	.....	63.3	....do....	.....	
	71.4	....do....	.....		5.....	68.0	....do....	.....	
2.....	.....	.....	59.2	Do.	6.....	76.9	Bushnell	.....	
3.....	71.2	Smith...	.....		7.....	60.0	Low....	.....	

Allowing for inexperience with the method, it is evident that the starch found ranges between 60 and 75 per cent of the cereal added. This means that a sausage having 3 per cent cereal would show from 1.80 to 2.25 per cent starch. This is too wide a margin. The following comments were received from analysts:

L. S. Bushnell, Kansas City, Mo.: The results obtained in the case of 0.5 per cent and 1 per cent bore no relation to the actual content. With the samples containing 5 per cent of cereal the results were fairly uniform. The chief difficulty with the method is that it is very troublesome to boil the sample enough to hydrolyze all the

starch and yet not to destroy any sugar. My conclusions are that this method promises to be the best we have (before Price's method was used), where the cereal amounts to about 5 per cent or greater, but for amounts lower than 1 per cent it is almost worthless.

Paul Rudnick, Chicago, Ill.: We met with difficulty in the boiling with sulphuric acid. Where results to the nearest per cent were sufficient, that is, where a difference in starch content of the cereal between 60 and 65 per cent was immaterial, we found the results by this method to agree quite satisfactorily with the factory formula.

At this stage the procedure of Price became known to the associate referee, and while it seemed to retain some of the long and tedious details of the present provisional method, the conditions were under control so well as to be preferable to that first tried. The method is this:

PRICE METHOD.

Treat in a 200 cc beaker 10 grams of finely divided meat with 75 cc of an 8 per cent solution of potassium hydrate in 95 per cent alcohol, and heat on the steam bath until all of the meat is dissolved. This will take from 30 to 45 minutes. Add an equal volume of 95 per cent alcohol, cool, and allow to stand at least one hour. Filter by suction through a thin layer of asbestos in a Gooch crucible; wash twice with warm 4 per cent potassium hydrate in 50 per cent alcohol and then twice with warm 50 per cent alcohol. Endeavor to retain as much of the precipitate in the beaker as possible until the last washing. Place the crucible with contents in the original beaker, add 40 cc of water and 25 cc of concentrated sulphuric acid. Stir during the addition of the acid and see that the acid comes in contact with all the precipitate. Allow to stand about five minutes, add 40 cc of water, and heat just to boiling, stirring constantly. Transfer the solution to a 500 cc graduated flask, add 2 cc of a 20 per cent solution of phosphotungstic acid, allow to cool to room temperature, and make up to mark with distilled water. Filter through a starch-free filter paper and after neutralizing determine the dextrose present in a 50 cc portion of the filtrate with Fehling's solution, using Low's method, Bulletin 107, Revised, page 241, for the determination of the copper in the cuprous oxid precipitate. Dextrose times 0.9 equals starch.

Known amounts of starch were added to meats by Mr. Price with the following results:

Results on meats by Price.

Starch added.	Starch found.	Error.	Starch added.	Starch found.	Error.
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
0.71	0.78	+0.07	3.64	3.70	+0.06
1.77	1.72	— .05	3.73	3.72	— .01
2.20	2.30	+ .10	4.36	4.26	— .10
2.22	2.31	+ .09	4.39	4.24	— .15
2.77	2.76	+ .01	6.26	6.26	± .00
3.04	3.12	+ .08			

Results on starch in cereals by Price method by other analysts.

Sample No.	Starch.	Analyst.
	Per cent.	
6	69.4	Smith.
7	70.6	Do.
8	64.8	Do.
9	69.1	Allcutt.
10	71.6	Do.
11	67.9	Smith.
12	63.1	Do.
Maximum	71.6	
Minimum	63.1	
Average	68.1	
12 samples corn flour:		
Maximum	71.8	Boyer.
Minimum	66.8	

With sausages containing known amounts of cereal the following results were obtained. The exact procedure of Price was used, except that the starch was filtered through an alundum crucible.

Results on sausages containing known amounts of cereal.

Starch in added cereal.	Cereal added.	Starch found.	Cereal found.	Error.	Analyst.
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
69.4	0.89	0.61	0.88	−0.01	Smith.
69.4	5.90	4.08	5.88	− .02	Do.
69.4	5.90	4.18	6.01	+ .11	Do.
70.6	.70	.48	.68	− .02	Do.
70.6	1.20	.83	1.18	− .02	Do.
70.6	2.09	1.61	2.28	+ .19	Do.
70.6	3.13	2.30	3.26	+ .13	Do.
70.6	5.74	4.04	5.72	− .02	Do.
70.6	6.24	4.37	6.19	− .05	Do.
70.6	2.50	1.67	2.37	− .13	Allcutt.
70.6	2.50	1.78	2.52	+ .02	Do.
70.6	5.00	3.45	4.89	− .11	Do.
70.6	5.00	3.36	4.76	− .24	Do.
71.6	.50	.45	.63	+ .13	Do.

Sausages containing spices but no cereals.

Analyst.	Starch found, per cent.
Smith	0.13
Boyer (average of 8samples, smoked domestic sausage)	.25
Marsh	.5
Allcutt	.19
	.27

COMPARISON OF THE TWO METHODS.

Mr. Boyer obtained within 0.25 per cent of the cereal added, the exact figure being uncertain because of variations in the starch content of the cereal. If 5 per cent of cereal is added, on a 67 per cent starch basis it would contain 3.35 per cent starch, and 3.60 per cent on a 72 per cent basis. The possible error here is greater than any error in the actual determination of the starch, which goes to show that this method is satisfactory so far as accuracy goes. This is especially important when less than 1 per cent of cereal is present, as, together with microscopical examination, it affords a means of finding a limit for the amount of starch that may normally be present because of such spices as pepper and ginger. Formulas published by Wilder [1] call for a maximum of 0.30 per cent spice starch, which accords well with the figures obtained.

The chief difference between the Low and Price methods is that the latter retains the treatment with potassium hydroxid of the provisional method. By using alcoholic potash instead of aqueous, Price shortened the process considerably, and the associate referee has been able to filter the starch through a specially made porous alundum crucible and make the 4 washings in 10 minutes, using suction. The increased accuracy due to the removal of the protein seems, therefore, to overbalance the time required, especially as without this treatment it is impossible to get good results when less than 1 per cent of cereal is present, owing to the action of the protein on the copper reagent.

The preliminary treatment with potash on pure cereals causes a loss of starch, amounting to as much as 3 per cent in some cases. This has not been definitely observed with meat products, owing perhaps to the neutralization of the potash by the meat, but it creates some uncertainty in calculating the starch to cereal, so that

[1] The Modern Packing House, 1905.

the figures given in this paper are subject to revision. The determination of the sugar properly belongs to sugar methods, hence will not be commented on.

The hydrolysis of the starch to sugar is very important, and the procedure of the Price method seems to be more uniform in its action than that of the Low method. Mr. Boyer's suggestion that even greater definiteness is needed may be a worthy one. It was noticed, however, that the temperature of the acid-sugar solution was only about 103° C. at the boiling point (water boiling at 98° C.), so that a few seconds more or less ought not to make much difference.

By the Low method sugar and other soluble carbohydrates would be estimated as starch, while with the other they would be separated in the first filtration.

The conclusions of the associate referee are that as the official methods are often used where accuracy is very important, and as for quick and rough work chemists are accustomed to shorten methods as they deem safe, it is preferable to adopt the Price method for this association. It is very much shorter and more dependable than the present Mayerhofer's method, the difficulties of which are almost insuperable.

The following comments were received from analysts:

E. A. Boyer, South Omaha, Nebr.: The copper can be as well determined by weighing as cuprous or cupric oxid. The results show perfect agreement between weighing as cupric oxid and the determination of the copper by Low's method, indicating that there is no contamination of the precipitate by mineral matter. The weighing as cuprous oxid has always given slightly higher results than the other methods, indicating that there is a slight contamination by organic matter. I believe the method of hydrolysis should be more definitely stated—that is, by using a thermometer, definite temperature for definite time.

Clarence T. Marsh, St. Louis, Mo.: We have found Dr. Price's method for the determination of starch in meat products satisfactory.

C. T. Allcutt. Kansas City, Mo.: The Low method would do where a variation of 20 per cent is allowable, but for close work the Price method is superior, and I have found it satisfactory for routine work. By allowing the potash solution to stand overnight and filtering the starch on a very coarse alundum filter that work is quickly done; moreover, the filtering of the cuprous oxid is almost instantaneous, while in the first method it is tedious and often impossible to filter the cuprous oxid, so that the difference between the two in point of time is small and not equal to the difference in accuracy.

W. H. Low: The Price method is not so simple as the other, and takes two or three times as long. By the other method the results have come within 0.25 to 0.50 per cent of the cereal supposed to have been added, which we call very satisfactory. We think you make a mistake trying for the most theoretically accurate process. It is much like determining the B. T. U. in a sample of coal within 5 of the truth, when the error in sampling may be 500 easily; it is not worth while taking the increased precautions. For investigation to find out where an error lies I am for the most accurate methods and instruments, but for commercial work I do not think it is necessary or desirable in all cases.

Ammoniacal Nitrogen in Animal Substances.

The referee in 1908 recommended the study of methods for determining ammoniacal nitrogen. Meanwhile there has been much work by biological and food chemists along this line. Many investigators, such as Folin, Pennington, Sherman, and Trescot, have shown that the provisional method of distillation in water solution with magnesium oxid causes hydrolysis of higher nitrogenous compounds that is much greater than the "ammonia" present before distillation. It has been shown that, other things being equal, sodium carbonate gives much the same results as magnesium oxid, the conditions which permit the hydrolysis being the high temperature of boiling and the presence of water. These authors have offered the following methods to overcome these difficulties:

Aeration method of Folin.—Passing a current of air through the sample solution to remove the ammonia. All heating is avoided and, as Folin states that ammonia is not split off from other compounds (in presence of water and sodium carbonate) below 37.5°, the method is very accurate.

Vacuum method.—As modified by Berg and Sherman it lowers the boiling point by distillation in a partial vacuum and by the use of strong alcohol, which also prevents hydrolysis by hindering the action of the water present.

Distillation at atmospheric pressure of a 60 per cent alcoholic extract of the flesh.—Principle same as that of the vacuum method except for the lowering of the air pressure.

The work this year consisted of a study of these three methods. Practically all points brought out in the literature were confirmed. As each collaborator could work best independently, no attempt was made to use the same samples. The data first given below are those obtained by the associate referee.

RESULTS OBTAINED BY W. B. SMITH.

Provisional method.—Some results were obtained by the present method of distillation with water and magnesium oxid, for comparison with other methods to be described. All figures in this paper are given as per cent of ammoniacal nitrogen.

Sample.	Method.				
	Water distillation.	60 per cent alcohol.	95 per cent alcohol.	Vacuum.	Aeration.
Sour cured beef	0.0465 .0499	0.0150 .0214	.....	.....	.....
Stale sausage	.062 .044	.027	.....	.....	.....
Very fresh beef	.031	.....	0.0098	0.0098	0.0083

As the higher figures given by the provisional method can only be due to hydrolysis of higher compounds, the sooner this method is dropped the better.

Richardson method.—At this time the method of Richardson appeared advantageous. This consisted in extracting 100 grams of sample three times with 150 cc portions of 60 per cent alcohol, and distilling the extract with magnesium oxid. The results are nearly true, but the extraction is tedious and unnecessary, and on further extraction of the flesh from 0.003 to 0.006 per cent nitrogen in addition could be obtained.

In order to show that none of the ammonia remained behind in 60 per cent alcoholic distillation, a solution of ammonium chlorid made up to contain 0.0206 gram of nitrogen was distilled first without meat, but with alcohol and magnesia.

	Nitrogen, gram.
First distillation of 200 cc gave	0.0200
Second distillation of 50 cc gave	.0006
Third distillation of 50 cc gave	.0000
Nitrogen recovered (against 0.0206 gram added)	0.0206

One hundred grams fresh beef were then treated to a first extraction and distillation, which removed 0.016 per cent of nitrogen; 0.0206 per cent of nitrogen as ammonium chlorid was then added to the residue, and two distillations made.

Distillation No.	Amount of distillate.	Nitrogen obtained.	Nitrogen in blank on meat.	Ammonium chlorid, nitrogen.
	cc.	*Per cent.*	*Per cent.*	*Per cent.*
1	200	0.0234	0.0026	0.0208
2	100	.0010	.0011	— .0001
		.0244	.0037	[1].0207

[1] Recovered (against 0.0206 added).

This would seem to be additional proof that the high results obtained by the water distillation are due to decomposition during the operation. Some of the figures by Richardson's method follow:

Results on nitrogen in meats by Richardson method.

Sample.	Extraction—			
	1	2	3	Total.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Stale deviled ham	0.0185	0.0035	.....	0.0220
	.0169	.0028	.....	.0197
Canned tripe, when opened	.0121	.0021	.....	.0142
	.0129	.0021	.....	.0150
Canned tripe, 2 days after being opened	.0137	.0020	.....	.0157
Fresh chopped beef:				
24 hours in ice box	.0113	.0029	0.0023	.0165
48 hours in ice box, good	.0164	.0026	.0023	.0213
72 hours in ice box, dark	.0182	.0029	.....	.....
96 hours in ice box, stale odor	.0203	.0028	.0025	.0256
144 hours in ice box, spoiled	.0214	.0043	.0020	.0277
192 hours in ice box, rotten	.0853	.0161	.0066	.1080

While these results show fair agreement between duplicates and also show progressive decomposition fairly well, there is no end to the reaction, so that the amount of distillation is purely arbitrary. Some irregular results led to the discovery that a variation in the strength of alcohol used caused errors greater than the natural increase in ammonia in a sample kept at 12° C. overnight, 0.002 to 0.005 per cent. For example: 25 grams of beef distilled directly with magnesium oxid and alcohol gave the following results:

Percentage of nitrogen found in 25 grams of beef distilled directly with magnesium oxid and alcohol.

Condition of sample.	Distillation No.	Amount of distillate.	Nitrogen.		"Cleavage ammonia."
			60 per cent alcohol.	95 per cent alcohol.	
		cc.	*Per cent.*	*Per cent.*	*Per cent.*
Nearly fresh	1	200	0.0136	0.0118	0.0018
	2	100	.0029	.0025	.0004
	3	100	.0019	.0015	.0004
			.0184	.0158	.0026
Stale	1	200	.0281	.0169	.0112
	2	100	.0041	.0024	.0017
	3	100	.0021	.0013	.0008
			.0343	.0206	.0137

As the boiling points of 60 per cent and of 95 per cent alcohol differ by only 2 degrees, the excessive hydrolysis with 60 per cent must be due to the water and not to the heat. While with fresh flesh the tendency to hydrolysis, though marked, is not perhaps insuperable, with aging meat the use of weak alcohol renders this method useless for accurate work. On the other hand, a little water, whether present in the sample or added, appears necessary in order to permit conjunction of the ions. When samples were too dry the ammonia was evolved with difficulty.

Reagents: Both sodium carbonate and magnesium oxid are used as alkali, while many authors recommend the use of salt to prevent dissociation. The results obtained by comparative tests of these follow:

Results on nitrogen when sodium chlorid was used.

Kind of sample.	Distillation No.	Amount of distillate.	Nitrogen.			"Cleavage ammonia."
			Magnesium oxid.	Sodium carbonate.	Sodium chlorid and sodium carbonate.	
		cc.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Stale beef	1	200	0.0178	0.0178	0.0176	0.0002
	2	100	.0026	.0026	.0028	— .0002
	3	100	.0015	.0019	.0012	.0007
			.0219	.0223	.0216	.0007
Sour sausage	1	200	.....	.0314	.0296	.0018
	2	100	.....	.0017	.0018	— .0001
	3	100	.....	.0008	.0011	— .0003
			.....	.0339	.0325	.0014

While the variations in these results were small enough as to be within the errors of separate determinations, the evidence seems to point to the usefulness of salt. The same result was found by Folin and by Berg and Sherman, using the aeration and vacuum methods, respectively. In my subsequent work by all methods salt was used, and generally sodium carbonate, although sometimes magnesium oxid.

It was decided from these tests that the following method, which was very quick and easy of operation, was accurate enough to show changes occurring in flesh kept a few hours at 10 to 15° C.:

Distill 200 cc of a solution made of 25 grams of flesh, or of a solution of the sample containing not over 20 cc of water, 5 grams of sodium chlorid, 1 gram of sodium carbonate, and 450 cc pure ethyl or methyl alcohol, make distilling mixture up to volume so as to keep the strength of the alcohol as high as possible, and distill two more portions of 100 cc each.

Later the procedure (described by Mr. Loomis in the following section) of using a current of alcohol vapor came to notice and was found to give even better results, as far as has been determined, than the above method, probably because direct heating of the flesh mixture is avoided. It was therefore used in the later work.

Detailed results by this method, besides those already given, follow:

While the history of the first seven samples was unknown, except No. 1, from the neck of a cow, an observer would arrange them on visual inspection much as they are arranged in the table. Sample 4 increased 0.0015 per cent overnight. The remainder of the determinations were on manufactured products, mostly showing high ammonia from processing rather than from decomposition, except No. 10, which was distinctly sour.

Ammoniacal nitrogen by alcoholic distillation.

Sample.	Nitrogen.			
	First distillation (200 cc).	Second distillation (100 cc).	Third distillation (100 cc).	Total.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1. Chopped beef, 24 hours dead (sodium carbonate)	0.0082	0 0013	0.0002	0.0097
2. Chopped beef, fresh (magnesium oxid)	.0085	.0015	.0009	.0109
Duplicate	.0085	.0016	.0014	.0115
3. Chopped beef, fairly fresh (sodium carbonate)	.0094	.0034	.0016	.0144
4. Chopped beef, fairly fresh (magnesium oxid)	.0132	.0017	.0009	.0158
4a. Same, 24 hours later (magnesium oxid)	.0145	.0015	.0013	.0173
5. Chopped beef, good but not fresh (sodium carbonate)	.0141	.0024	.0008	.0173
6. Chopped beef, dark color, old (magnesium)	.0168	.0025	.0015	.0208
7. Chopped beef, dark color, old (magnesium)	.0221	.0019	.0013	.0253
Same, one distillation nearly to dryness				.0260
8. Old summer sausage (magnesium)	.0249	.0021	.0015	.0285
8a. Same, 1 day later (magnesium)	.0233	.0033	.0016	.0282
8b. Same, 2 days later (magnesium)	.0244	.0024	.0016	.0284
9. Sausage, stale but not spoiled (sodium)	[1].0269	.0014	.0008	.6291
10. Sausage, spoiled (sodium)	.0296	.0018	.0011	.0325
	[1].0283	.0015	.0008	.0306
11. Meat extract (sodium)	[1].181	.011	.003	.195
12. Meat extract (sodium)	.193	.017	.002	.212
13. Meat extract (sodium)	.277	.005	.002	.284
14. Canned salmon, just opened (sodium)	[1].0432	.0006	.0005	.0443
14a. Canned salmon, 20 hours on ice (sodium)	[1].0448	.0005	.0003	.0456
15. Fresh salmon (sodium)	[1].0104	.0006	.0005	.0115

[1] Vapor process.

The determinations marked with the superior number were performed by the vapor process, which compares, according to Sample 10, extremely well with the ordinary method. The only desideratum in the alcoholic method is that an end point might be reached. Duplicates appear good, and slight changes in progressive decomposition are easily indicated. It seems gratifying, moreover, that a sample (13) showing 0.277 per cent of nitrogen in the first distillation should fall to 0.002 per cent on the third. From all of the results by the referee it seems true that the totals just given represent no more, or possibly slightly less, than the amount of ammonia present, a supposition borne out by figures by the vacuum and aeration methods. It will be noticed that when Sample 7 was treated by the regular method, only 0.0253 per cent was obtained, while distillation of the original 450 cc nearly to dryness gave 0.0260 per cent, a slight difference, it is true, but indicating that the process was not carried too far in the former case. But as there is probably some hydrolysis, and as the extremes in the fourth distillation would cause a relative variation in the totals, within the same class of samples, of much less than 0.001 per cent, there seems no particular advantage in carrying it further. Besides, the error in titrating 100 cc of distillate would add to its impracticability, one drop of tenth-normal soda being equivalent to 0.00028 per cent of nitrogen. Possibly the vapor process may be capable of completion, practically no ammonia being found in some cases on the third distillation.

The method just described was compared with the aeration (Folin's method, modified) and the vacuum distillation methods, details of which follow:

Aeration method.—Arrange five vessels in a series, as follows: (1) A bottle of sulphuric acid, with a Hopkins safety bulb, to purify the entering air. (2) A 1,000 cc flask containing 25 grams of sample, 250 cc of water, 5 grams of sodium chlorid, and 1 gram of sodium carbonate. Alcohol may be added to prevent foaming. (3) A 250 cc safety flask. (4) A cylinder, fitted with a Folin absorption tube, containing tenth-normal sulphuric acid. (5) A 100 cc safety flask.

Connect the last flask to an air pump powerful enough to draw the ammonia over into the standard acid. Alcohol may be substituted in whole or in part for the water, to assist a weak air current. The standard acid is titrated at intervals of an hour until no more ammonia comes over. Methyl Red, Cochineal, or Congo Red may be used in aqueous solutions, Methyl Red or Cochineal in alcoholic.

Vacuum method.—Distill 25 grams of meat, 250 cc of alcohol, methyl or ethyl, sodium carbonate or magnesium oxid, and salt under reduced pressure, at about 60° C. until the residue in the flask is about 50 cc.

The aeration and vacuum methods are capable of great accuracy, but as the vacuum method is not needed no attempt has been made to perfect it. More care and experience are needed for its successful operation than for the other methods, and unless the conditions are perfectly controlled the results are often high, as will be noticed in one or two of the following cases. The figures obtained by these methods are here given, together with the amounts found by the alcoholic method, taken from the results of the preceding method.

Ammoniacal nitrogen in meat.

Sample.	Nitrogen.		
	Aeration method.	Vacuum method.	Alcoholic distillation method.
	Per cent.	*Per cent.*	*Per cent.*
1. Beef, 24 hours on ice	0.0083	0.0098	0.0097
3. Beef, fairly fresh	.....	.0136	.0144
4. Beef, fairly fresh	.....	.0168	.0158
4a. Same, 24 hours later	.0151	.....	.0173
5. Beef, good but not fresh	.0174	.0174	.0173
Duplicate	.....	.0179	.....
5a. Same, 24 hours later	.0176	.0190	.....
6. Beef, dark color, old	.....	.0210	.0208
7. Beef, dark color, old	.....	.0235	.0253
Duplicate	.....	.....	.0260
9. Sausage, stale but not spoiled	.0291	.....	[1] .0291
12. Meat extract	.22	.....	.212
13. Meat extract	.286	.....	.284
14. Canned salmon, as opened	.0442	.....	[2] .0443
14a. Same, 20 hours later	.0465	.....	[2] .0456
15. Fresh salmon	.0120	.....	[2] .0115

[1] Vapor process. [2] Vapor.

One or two of the results show quite a variation between different methods, but upon comparison with those obtained by the provisional method it seems remarkable that in the presence of easily hydrolyzed nitrogenous compounds the evolution of ammonia should be so uniform. It shows that this figure represents closely the most loosely bound nitrogen, that known as ammonia, and that it may be determined with an accuracy capable of showing changes occurring in flesh kept a few hours on ice.

Folin found 0.0005 per cent of nitrogen in arterial blood, 0.0010 per cent in venous blood, and 0.0057 per cent in dog liver tissue. The referee found 0.0073 per cent (vacuum method) in beef muscle 4 hours after killing and an average of 0.0093 per cent 24 hours after death.

The following summary shows the amount of ammonia obtained by three persons using two methods, on three kinds of fresh flesh, entirely independent of each other:

Ammonia.

	Aeration method.	Alcoholic distillation method.	Analyst.
	Per cent.	*Per cent.*	
Poultry flesh	0.011 to 0.012	.....	Pennington.
Salmon flesh	.....	0.011 to 0.013	Loomis.
Salmon flesh	.012	.0115	Smith.
Beef flesh	.....	.010 to .011	Do.

The aeration method gives good results, the only objection to it being the apparatus required in order to perform several determinations at the same time, the length of the operation, and the foaming complained of by some. Very little foaming was observed with a sample of fresh salmon examined, and it may be that the difficulty is caused by using magnesium oxid instead of sodium carbonate, which foams much less, and by using insufficient alcohol. On the other hand, the alcohol-vapor method is extremely quick and easy to handle, needs little apparatus, and gives apparently as good results as the aeration. It would seem advisable, then, to give some recognition to this method.

It was noticed that with samples high in ammonia the end point by the aeration method was in some cases as difficult to reach as in the distillation method. The chief advantage of the aeration method is that, heat being absent, an excess of water does no harm.

Indicators and standard solutions.—For atmospheric distillations tenth-normal hydrochloric or sulphuric acids were used and titrated against tenth-normal sodium hydroxid, with Cochineal or Methyl Red. For the vacuum or aeration methods sulphuric acid must be used. Congo Red is very good for titrating ammonia, but the presence of much alcohol renders it unreliable. A blank was run on all the reagents used.

While for blood, living tissues, and milk Nessler's reagent may be as indispensable as it is for ammonia in water, for the larger amounts present in meat titration appears simpler and perhaps even more accurate.

References: Folin, Zts. physiol. Chem., 1902, *37*:161.
Richardson and Scherubel. J. Amer. Chem. Soc., 1908, *30:* 1515.
Berg and Sherman. J. Amer. Chem. Soc., 1905, *27:* 124.
Pennington and Greenlee. J. Amer. Chem. Soc., 1910, *32:* 561.
Trescot. U. S. Dept. Agr., Bureau of Chemistry Bul. 132, p. 20.

RESULTS OBTAINED BY H. M. LOOMIS.

Determination of ammoniacal nitrogen in salmon products.—Owing to the excessive foaming, I could not use the aeration method on fresh salmon; therefore confined my work to two methods, that of distillation at atmospheric pressure with 95 per cent alcohol, and one which I have been trying, and which I have modified as follows:

Alcohol vapor-current method.—Mix 25 grams of finely chopped sample of fish with 5 grams of freshly ignited magnesium oxid and 100 cc 95 per cent alcohol, in a 500 cc flat-bottom Florence flask, and distill in a current of vapor from boiling 95 per cent alcohol. Distill 400 cc into tenth-normal acid. Change the receiver and distill 100 cc more, and repeat this process until not more than 1 drop of tenth-normal acid is required to neutralize the 100 cc distillate.

Of the results following, all the samples of canned salmon were apparently in very good condition, with the possible exception of the one designated as No. 5. The agreement between duplicates was very good. After distilling 200 cc, the "ammonia" dropped off much more suddenly, in a second and third distillation of 100 cc each, in the vapor process than in direct distillation with alcohol. I am unable to explain why the vapor figures on Samples 7 and 8 should be higher than the corresponding values by the other method.

Nitrogen results on salmon.

Sample.	"Ammoniacal" nitrogen.		Sample.	"Ammoniacal" nitrogen.	
	Alcoholic distillation method.	Alcohol vapor current method.		Alcoholic distillation method.	Alcohol vapor current method.
Fresh salmon:[1]	*Per cent.*	*Per cent.*	Canned salmon—Continued.	*Per cent.*	*Per cent.*
No. 7. Sockeye, Puget Sound, 15543–D.	0.0111 .0131	0.0205	No. 2. Pink, Alaska, 15532–D.	0.0402 .0406	.....
After keeping 6 days in ice chest	.0222	.....	No. 3. Cohoe, Alaska, 15560–D.	.0496 .0497	.....
After keeping 40 hours longer in laboratory at 70° to 80° F.	.0729	.0760	No. 4. Red, Alaska, 15534–D.	.0461 .0450	.....
No. 8. Steelhead, Puget Sound, 15544–D.	.0130 .0141	.0218	After keeping 6 days in ice chest	.0499	.....
After keeping 5 days in ice chest	.0240	.0236	No. 5. Sockeye, Puget Sound (old stock), 15533–D.	.0437 .0437	0.0410
Canned salmon:					
No. 1. Chum, Alaska, 15563–D.	.0562 .0564	.0559 .0555	No. 6. Sockeye, Puget Sound, 15572–D.	.0399 .0408	.0348

[1] Analyzed within 24 hours after leaving the water.

RESULTS OBTAINED BY OTHER ANALYSTS.

M. E. Pennington, Philadelphia, Pa.: The figures offered by M. E. Pennington have been published, the following being selected as valuable for our purpose:

Nitrogen results on chicken flesh.

Sample.	Nitrogen.	
	One distillation: water, or provisional method.	Aeration method.
	Per cent.	*Per cent.*
No. 299. Chicken flesh 2 years in storage	0.036	0.023
No. 304. Chicken flesh 6 years in storage	.109	.058
No. 308. Chicken flesh 24 hours at 0° C	.041	.012
	Aeration method.	
	Magnesium oxid.	Sodium carbonate.
	Per cent.	*Per cent.*
No. 308. Chicken flesh	0.012	0.011
No. 309. Chicken flesh	.011 .011	.011 .011
No. 310. Chicken flesh	.012 .012	.012 .010
No. 311. Chicken flesh	.014 .014	.014 .012
No. 312. Chicken flesh	.016 .016	.016 .016

The rate at which the ammonia was evolved rapidly decreased until at the end of 4 or 5 hours none was evolved during an hour.

The following amounts of ammoniacal nitrogen were evolved from poultry flesh by the aeration method:

	Per cent nitrogen.
Perfectly fresh	0.011 to 0.012
Kept cold 4 to 9 days	.014 to .019
Cold stored 1 year	.019
Cold stored 2 years	.023 to .032
Cold stored 6 years	.058

Miss Pennington stated further: We have quite discarded the distillation method, for chicken flesh at least, in favor of the aeration method. The methods of Richardson (water distillation and distillation of a 60 per cent alcoholic extract of meat) were universally unsatisfactory as compared with aeration. Prof. Folin confirms our observations in every respect. He stated that aeration was not available for use in fish flesh where there had been any heating, as in the preparation of canned fish. We had trouble also with dried eggs, but the distillation method was quite as unsatisfactory as the aeration. Apparently in this case at least it is not the method so much as the preparation of the product which causes trouble.

J. A. Emery and *R. M. Chapin, Washington, D. C.:* We believe that good results may be obtained by vacuum distillation, but the process requires proper apparatus and management. It demands considerable attention from an experienced worker, and does not seem so well adapted as the Folin method for handling a considerable number of simultaneous determinations. We agree that it is very desirable to replace the official by some better method. The Folin method has of late been considerably employed in this laboratory. We are inclined to prefer sodium carbonate for the necessary alkali. The addition of salt does no harm, and while it probably is not necessary in all cases, yet it may be in some and in view of the uncertainty the safer procedure is provisionally to retain it.

H. C. Sherman, New York, N. Y.: Our vacuum method for ammonia in milk consisted essentially in the use of an amount of methyl alcohol equal in volume to the aqueous solution, and the use of sodium carbonate as alkali, with saturation with sodium chlorid. I have no doubt that Folin's method properly carried out with a strong current of air is quite as accurate and probably more so. It seems to me very desirable that the use of magnesium oxid with simple distillation should be dropped from the official methods as early as possible and some more accurate method such as that of Folin prescribed in its place.

Paul Rudnick, Chicago, Ill.: In the case of eggs the foaming is, with Folin's method, so bad at times that it seems impossible to carry on the determination.

E. A. Boyer, South Omaha, Nebr.: I am convinced that the determination of ammoniacal nitrogen by the official method in substances containing loosely bound nitrogen is not only inaccurate but a waste of time. [Has used the aeration method successfully.]

W. C. Powick, Washington, D. C.: The present method causes progressive splitting off of ammonia from the more complex molecules, and has been abandoned for Folin's method. The latter gave 1.20 cc of tenth-normal ammonia when 1.19 cc had been added. With 50 grams of eggs the sodium carbonate could be increased to 5 grams without influence on the results. The alcohol seemed to be sufficient to preserve the sample.

Nitrates.

Caron and Raquet,[1] having claimed that the use of sulpho-salicylic acid in place of phenol-sulphonic acid for the colorimetric estimation of nitrates obviated the interference of chlorin, some work was done to test this statement. It was found that while as little as 0.5 mg of chlorin in the portion taken for evaporation caused

[1] Analyst, 1911, *36*: 85.

a serious error in the phenol-sulphonic method, rendering it necessary to precipitate the chlorin, 3 or 4 mg of chlorin had little effect on the color produced by the salicylic acid reagent, if fresh.

In order to test it further, several samples, chiefly curing solutions, were examined by this method and by the Schlösing-Wagner gasometric method. A few tests by the phenol-sulphonic method, without precipitating the salt, gave the usual erratic and valueless figures. The sulpho-salicylic method is as follows:

Sulpho-salicylic method.—Evaporate the solution, containing about 0.1 mg of nitrate nitrogen to dryness on the water bath, after the addition of 1 cc of a 1 per cent solution of sodium salicylate, freshly prepared. Mix the residue with 1 cc of concentrated sulphuric acid, stir well, and allow to stand two minutes. Add 10 cc of water and an excess of 30 per cent potassium hydroxid. Decolorize the solution with alumina cream if sugar or other charred bodies are present, filter, and make to 100 cc, then compare with a standard solution prepared in the same way.

The figures obtained by the two methods are given in the accompanying table.

Cooperative work on meat-curing pickles and meat.

Sample.	Schlösing-Wagner method.	Sulpho-salicylic method.	Analyst.
1. New pumping pickle	1.81	1.76	C. T. Allcutt.
	1.81	1.74	E. A. Boyer.
	1.82	1.67	Do.
	....	1.72	W. B. Smith.
Average	1.81	1.72	
2. Used curing pickle	.48	.41	C. T. Allcutt.
	.39	.37	E. A. Boyer.
	.41	.39	Do.
	....	.40	W. B. Smith.
	....	.36	Do.
	....	.39	Do.
Average	.43	.39	
3. New pumping pickle	4.02	....	C. T. Allcutt.
	3.97	....	Do.
	4.10	3.37 (?)	E. A. Boyer.
	4.04	3.46 (?)	Do.
	....	4.13	W. B. Smith.
	....	4.00	Do.
Average	4.03	4.06 (?)	
4. New pumping pickle	3.40	3.34	C. T. Allcutt.
	....	3.42	Do.
	3.54	3.33	E. A. Boyer.
	3.49	3.46	Do.
	....	3.38	W. B. Smith.
	....	3.44	Do.
Average	3.48	3.39	
5. New curing pickle	....	.98	C. T. Allcutt.
	1.05	1.11	E. A. Boyer.
	1.06	1.11	Do.
Average	1.05	1.07	
Chlorin precipitated	....	1.05	W. B. Smith.
6. Chipped dried beef	.11	.03	E. A. Boyer.
	....	.06	W. B. Smith.
Average	.11	.04	
Chlorin precipitated	....	.12	Do.
7. Pumping pickle	1.74	1.75	
8. Pumping pickle	1.82	1.73	
		1.55	
9. Pumping pickle	2.75	2.56	
		2.53	
10. Pumping pickle	4.70	4.49	
11. Pumping pickle	3.47	3.24	
12. Pumping pickle	2.01	1.78	
	2.01	....	

Allcutt. Smith.

It is evident that in pumping pickles containing several per cent of saltpeter and up to 25 per cent of salt the new method gives results from 3 to 7 per cent too low. With this class of products its shortness makes it of much value. With pickles containing less than 2 per cent of saltpeter the error becomes larger, the relative amount of salt generally being greater, so that for accurate work it is necessary to precipitate the chlorin. Only one sample of meat was tested at this time, but from previous experience there can be no doubt that the results are fully representative of cured meats in general. About 0.11 per cent of nitrates as saltpeter was present, while by this method only 0.03 to 0.06 per cent was obtained. Meats are cured with small amounts of saltpeter, and this is largely converted into other forms, while the chlorin is unchanged. The maximum effect of the chlorin is therefore experienced when nitrates are determined in meats, rendering the method useless for this purpose unless the chlorin is precipitated.

The advantages of the sulpho-salicylic method are:

(1) The reagents are easily made up.

(2) The method is short.

(3) The color produced is much stronger than by the phenol-sulphonic method.

(4) Much more chlorin may be present without seriously affecting the results.

The disadvantages are:

(1) The sodium salicylate solution must be fresh.

(2) In the majority of cases the chlorin must be precipitated, when the advantage over the phenol-sulphonic method disappears.

No recommendation is made in regard to nitrates.

Recommendations.

It is recommended—

(1) That on page 106 of Bulletin 107, Revised, under "XVII. Methods for the analysis of meat and meat products. 1. Identification of species—Provisional," fourth line, after "melting point," "melting point of stearin by Belfield-Emery method" be inserted.

(2) That Mayerhofer's method, modified (Bul. 107, Rev., p. 109), be replaced by Price's method (see p. 97) for the detection or determination of starch.

(3) That Folin's method by aeration, as modified (see p. 99), be substituted for the method for ammoniacal nitrogen in animal substances under "(e) Ammonia.—Provisional," Bulletin 107, Revised, page 115.

(4) That the foaming difficulty of the aeration method be further studied.

(5) That the following distillation method be studied: Mix 25 grams of the fine sample with 5 grams of salt, 1 gram of sodium carbonate, and 100 cc of alcohol, in a 500-cc flask, and pass through it a current of vapor from boiling alcohol. Distill a 200-cc portion and titrate and then treat similarly two 100-cc portions. The last portion should contain very little ammonia.

PRESIDENT'S ADDRESS.

By H. J. Patterson.

Members of the Association of Official Agricultural Chemists:

The example set by my twenty-eight predecessors makes it incumbent on me to say a few words to you on this occasion. Each year it becomes more and more difficult or perhaps I would better say that it seems less and less necessary for the president to perform this duty, owing to the fact that the work of the association has already been so well mapped out and covered and the merits and defects so thoroughly appreciated.

It was my privilege to become affiliated with this association at its fifth session in 1888, and I have attended all but three of the meetings since that time. There were 26 chemists present at that fifth meeting, 9 of whom have passed to their final rest

and 9 still continue their interest in the association and attend the meetings quite regularly, but only 3 are present at this convention. In recent years my duties have not permitted me to do the active work for the association that I should like to have done, but I assure you that I have never lost interest in your work and that I fully realize that I have lost much by not being able to come into closer contact with the details of your operations. At this juncture I must pause to express to you my appreciation of the honor you have done me by giving me an opportunity to serve as your presiding officer. I doubt if the honor was merited. I wish to congratulate the association on its composition, its character, and its work. I have come into contact with a good many organizations and associations, and to me this association is unique in that it is made up of persons who have had nearly the same kind of training and who have almost identical purposes and desires. Consequently, when they meet they get down to work immediately, pull together in good team fashion, and get results, accomplishing much more than many associations do in twice the time. The results obtained are not only great in quantity, but the high quality has made their methods the world's standards.

I doubt if many of us, particularly the younger members, fully appreciate the influence which this association has had directly and indirectly upon the agricultural development of our country. Many persons look upon the official agricultural chemist as a control or police chemist whose chief function is to safeguard the public from fraud and other impositions. While this is an important function that has been worth many thousands of dollars annually, yet great value has come from the constructive and research work conducted by our members, which was largely made possible through the methods devised by this association.

The pioneer work of Johnson, Atwater, Brewer, Goessmann, Lupton, and Caldwell paved the way and created the demand which led to the establishment of the first experiment stations in the United States.

This work also paved the way for fertilizer inspection, which soon necessitated the formation of this association, which was organized several years before the National Government provided for experiment stations in each State, and whose influence was a potent factor in molding the sentiment which resulted in the passage of the Hatch Act.

Among the early members whose work was influential in creating a demand for scientific help in farming may be mentioned Wiley, Jordan, Armsby, Babcock, Dabney, Jenkins, Frear, Scovell, Myers, Stubbs, and Kedzie.

Nearly 40 per cent of the directors selected to initiate the station work in 1888 were taken from the members of this association, and I believe every station had a chemist on its staff from the first. This condition did not obtain with any other class of scientists, and even to-day chemists continue in the majority. In addition to the research work conducted by the chemists there is a large call for chemical assistance from other specialists who use this science as a handmaid in their work. Chemistry is also frequently needed as the court of last resort for the ultimate solution of many of the difficulties met by investigators in other fields.

A consideration of these few facts is sufficient to impress upon us the influence which the members of this association have exerted in the development of our agricultural institutions. The chemists may also take some credit for the new life which was infused into the agricultural colleges through the development of the experiment stations.

No argument is necessary to convince us that the agricultural colleges and experiment stations of the United States have exerted an immeasurable influence in developing our agricultural resources and in keeping the average productiveness up to the present level. The failure of this influence to be more potent has been due to the difficulties experienced in getting the results of research to the farmers who do not observe, read, or attend farmers' meetings and who seldom go far from home to see new things.

While we glory in the record of our association and take pride in doing honor to the men who have been influential in bringing the work to its present standard, we should not lose sight of the duty that devolves upon us now to see that we continue to follow a policy that will enable us not only to maintain our present position but, if possible, to expand so as to make the chemists of the future still greater factors in our agricultural development. By so doing we shall strengthen the influence of this association. To accomplish this end it will be necessary for many laboratories to become less commercial and more devoted to research.

The high pressure and limited funds under which most laboratories operate frequently develop a routine chemist instead of an investigator. Every member of the chemical force, from highest to lowest, in the various institutions represented in this association, should engage in research, if the greatest good is to be accomplished. Members of every laboratory should be encouraged to converse freely with one another concerning their problems. Those who have students specializing in chemistry should encourage them and give them an opportunity to become acquainted with all the work in progress in the laboratory. Whenever possible utilize these students' services in connection with the investigations being pursued. Every professor and his assistants should form a family party and all mix example with precept, thus creating a chemical atmosphere and inspiration for the development of latent talents. It will make fewer machine and more inventive chemists, not making investigators out of all students of course, for it is the desire of many simply to *do*, while the true investigator's chief desire is to *know* rather than to do. Such a policy, however, would do much to segregate the functions of the college from those of the professional school. For the greatest development of research laboratories it would be well whenever possible to have double the number of assistants needed for the routine and inspection work and allow them one-half of their time to themselves for study and research.

I realize that in practice it is hard to carry out this plan to-day, owing to the great demand for men with a college training at salaries which are high as compared to those paid 10 to 15 years ago, and at a wage that makes most men feel that they can no longer give up time and money for further study. Nevertheless, I believe that it should be the policy of most laboratories to offer such an opportunity and I feel sure that the ultimate result will be good and that under existing conditions we shall frequently find young men and young women anxious for such opportunities.

The question of the salary to be paid under such circumstances is difficult as well as delicate, but in general it may be said that as opportunities will be part of the pay the salary should be only sufficient to live unmarried in comfort and not great enough to induce a man to spend his life in a subordinate place. The demand for such places will grow when people fully realize that the opportunities and salaries are proportionately greater for broadly trained and seasoned investigators or inventive chemists. With the rapid development which is going on in every field that now uses chemists and the continual opening of new fields, chemists need not fear growing old in subordinate positions or of growing weary or sick with hope deferred.

An extension of this plan in the United States Government laboratories would prevent to some extent their drawing so heavily on the State laboratories and might under some circumstances turn the tide the other way.

In the development of research laboratories and the training of investigators or in the selection of assistants the obtaining of a degree or the degree possessed should not be considered as the only measure of value. This association should use its influence to have the degrees granted in chemistry mean more than they do in some instances. America needs a standardization of degrees. A degree should represent more than a parrot-like ability to repeat what has been heard or read. It should indicate a capacity for research and what a man can do rather than what he knows. The higher the degree the greater should be the development of the power to correlate facts and measure their value.

In the development of research chemists more attention should be given to having some men acquire a good working knowledge in some allied science which is freqently needed in order that one man may be able to handle the whole of a problem instead of as at present having to intrust part of it to some one else who may fail to sympathize with the work in hand or with the objects sought. For instance, in many investigations which stations have under way a working knowledge of bacteriology or plant pathology with chemistry is very helpful. There are many other combinations that would prove helpful. In any plan which is adopted, having for its object the stimulation of research and the development of investigators, it must be expected that all will not develop equally or with the same tendencies. They will, however, develop, and in doing so will divide themselves naturally into about three classes:

First. The routine chemist, who lacks initiative and who does not want responsibility. Most of this class will prove to be trustworthy, hard-working, and good analysts.

Second. The managing commercial or factory chemist, who has the ability to apply his knowledge practically and can manage men.

Third. The research chemist, who delights in new problems, whether original with himself or suggested by others.

Another condition which should be overcome if the highest standards and the greatest usefulness is to be maintained for the association is the organization of laboratories or chemical departments so that the time of the men of talent is not wholly consumed for administrative purposes or for dispensing information to the public.

The rate of compensation for the talented investigator should be on a plane with that of the administrator's office and thus prevent the loss of investigators for financial reasons.

There has been a steady improvement from year to year in the association's methods of analysis; while in most cases those in use may be considered quite satisfactory, it is probable that most of them can be still further improved. The importance of the official methods both for control and for research work would seem to warrant almost every laboratory in devoting more time than they do at present to this class of studies, and especially to the analysis of cattle foods. Attention has been repeatedly called to the need for a better division of the food constituents and better methods for analyzing cattle foods; yet it is doubtful if any real progress or improvement has been made in the last 15 years. Methods now in use put into the same class too many components of a widely different character and feeding value. Methods for the analysis of cattle foods are needed which will put compounds of like feeding value into the same class and which will be fairly simple, rapid, and accurate. These are difficult specifications to fill, but when we realize that good methods are the foundation for good investigations and good practice it behooves all to be alert and active in perfecting these tools.

It would be ungrateful if in this connection mention were not made of the services rendered this association by Dr. Wiley, who filled the office of secretary for 22 years. It was through his instrumentality that the United States Department of Agriculture provided for years a meeting place for the association and furnished all the needed facilities for recording our deliberations and, more than all, for the publication of our annual reports. Without the faithful services of the secretary and the facilities given by the United States Department of Agriculture we should have fallen far short of what has actually been accomplished by the Association of Official Agricultural Chemists. This association owes much to Dr. Wiley and we should provide for some special or emeritus membership whereby we may continue to profit by his counsel and do ourselves the honor of having him closely associated with us.

The appointment of the following committees was announced:

Committee to draw up resolutions to be presented to the State agricultural experiment stations for the purpose of promoting the association work.—B. L. Hartwell, of Rhode Island; R. E. Doolittle, of the District of Columbia; P. F. Trowbridge, of Missouri; E. F. Ladd, of North Dakota; and W. A. Withers, of North Carolina.

Committee on recommendations of referees and revision of methods.—P. F. Trowbridge, chairman; *Subcommittee A*, A. J. Patten (1918); *Subcommittee B*, F. W. Woll (1918), P. F. Trowbridge (1916) to replace H. E. Barnard (1916); *Subcommittee C*, L. M. Tolman (1918), H. E. Barnard (1916) to replace P. F. Trowbridge (1916).

Adjourned.

TUESDAY—AFTERNOON SESSION.

A paper on "The determination of starch in meat products," by E. M. Bailey and C. E. Shepard, presented by Mr. Bailey, has since been published in the Connecticut Agricultural Experiment Station Report of 1912, Part II, pages 122–124.

Mr. Willet M. Hays, Assistant Secretary of Agriculture, in an address to the association, spoke of the need for having biological problems taken up by men trained in physics and chemistry, and of the importance of favoring the advancement of pure science, now that the development of applied science is secured. He also brought out the necessity of introducing into the curriculum of our secondary schools simplified forms of the sciences relating to agriculture, the industries, and home making.

REPORT OF A SPECIAL COMMITTEE ON RESOLUTIONS.

The following report of a special committee on resolutions was unanimously adopted by the association:

Dr. Harvey W. Wiley, having been connected with the Association of Official Agricultural Chemists since its organization, and having been its honored secretary for nearly 30 years, it is eminently proper and a great pleasure for this association to place on record its appreciation of the great work which Dr. Wiley has done for the advancement of all the various lines of work undertaken by this association.

As a member of the association he has been the guiding spirit of all its activities, not only helping by his vigorous personality but by lending the aid of the many facilities which are afforded by the Bureau of Chemistry in developing and testing the various methods of analysis and other works which have been under consideration by the association during its entire history. His position as secretary of the association brought him into intimate contact with all the members, where his genial personality and unbounded enthusiasm, his far-sighted wisdom and unfailing good fellowship, promoted harmonious action.

The members of the Association of Official Agricultural Chemists desire to express their personal regret at the severance by Dr. H. W. Wiley of his active relation with the association and desire also to record their sense of the high value of his services to his country as a resourceful analyst, a writer of helpful scientific treatises, an effective organizer of scientific forces for practical purposes, and an able and vigorous exponent of high ideals of commercial ethics and good citizenship.

Your committee would recommend that Dr. Wiley be made honorary president of the association.

R. J. DAVIDSON,
E. F. LADD,
A. J. PATTEN,
JOHN PHILLIPS STREET,
Committee.

Adopted September 17, 1912.

REPORT ON FATS AND OILS.

By H. S. BAILEY, *Associate Referee.*

In 1891 Leffmann and Beam [1] published their method for the determination of the volatile acids in butter and allied products, substituting a glycerol for the usual alcoholic saponifying solution. Since then, glycerol has replaced the alcohol or water prescribed in the provisional method for the titer test [2] in the hands of a number of technical chemists. In 1910 C. V. Zoul [3] published the glycerol titer method used in the Procter & Gamble laboratories, and shortly after Campbell and Long [4] reported that a similar procedure had been used continuously in the laboratories of the Globe Soap Company since 1900. In the same journal [4] R. H. Kerr, of the Bureau of Animal Industry, mentioned the fact that he had already completed the development of a glycerol-potash titer method and thoroughly tested its accuracy when the work of Zoul appeared.

In spite of the fact that these methods have been criticized by H. Mielck [5] on the ground that the resulting fatty acids have not the same composition as those obtained when alcoholic potash is used as the saponifying agent, as shown by the lower iodin number, it seemed probable that glycerol could be used to advantage in the titer test.

The following methods and instructions were sent out to the collaborators, together with samples of cocoanut oil, cottonseed oil, house grease, oleo stearin, and tallow.

INSTRUCTIONS FOR COOPERATIVE WORK, 1912.

In taking up the study of the glycerol method for the saponification of fats in the making of the titer test, your associate referee suggests that the following methods be tried:

METHOD NO. 1.

Place 50 cc of high-grade glycerol and 20 cc of concentrated caustic potash solution (100 grams of potassium hydroxid dissolved in 100 cc of distilled water) in a liter flask and warm gently on an asbestos board over a low flame. When hot, add 50 grams of the melted fat and rotate the flask gently. Saponification begins at once and is complete when the mixture becomes perfectly clear and homogeneous. At this point remove the flame and add cautiously 500 cc of hot water. Then replace the flame and add sufficient dilute (1 : 3) sulphuric acid to decompose the soap. Boil until the layer of fatty acids is clear, then wash until free from mineral acids with repeated portions

[1] Analyst, 1891, *16* : 153. (Originally in pamphlet form, Philadelphia.)
[2] U. S. Dept. Agr., Bureau of Chemistry Bul. 107, Rev., p. 135.
[3] J. Ind. Eng. Chem., 1910, *2* : 479.
[4] J. Ind. Eng. Chem., 1911, *3* : 114.
[5] Chem. Zts., 1911, *35* : 668.

of hot water, and after filtering heat to 150° C. in a small casserole or beaker over a free flame, stirring constantly with a thermometer to avoid any local overheating, then proceed with the determination of the temperature at which the fatty acids crystallize, exactly as indicated at the bottom of page 135, Bulletin 107, Revised.

METHOD NO. 2.

Into a 750 or 1,000 cc Erlenmeyer flask pour 75 cc of a glycerol caustic potash solution made by dissolving 25 grams of caustic potash in 120 cc of high-grade glycerol. Heat until quite warm, then add from a cylinder or a pipette with a large outlet 50 cc of the fat or oil to be tested. The heating is continued until a homogeneous solution, almost entirely free from any frothing, results. Remove the flame and add, carefully at first, about 25 cc of cold water, then 40 cc of 1.21 specific gravity sulphuric acid. Replace the flame and continue the heating until the fatty acids have separated into a clear layer, when 400 cc of hot water should be added and the fatty acids washed and dried as directed in Method No. 1.

METHOD NO. 3.

Place 25 to 30 grams of the fat in a 400 cc beaker, cover with 100 to 150 cc of glycerol, and heat on a wire gauze after dropping in 4 or 5 grams of potassium hydroxid. Stir until the solution is complete and continue heating until the solution becomes homogeneous. Then pour the solution into 700 or 800 cc of hot distilled water, care being taken to mix the two gradually. Heat until perfectly clear. Acidify with dilute hydrochloric acid (1 :1) and heat until the fatty acids are entirely clear. Wash, dry and determine the crystallizing point as indicated in Method No. 1.

As a control on these three methods, each sample should be run by the official method, using either water or alcohol, as indicated on page 135 of Bulletin 107, Revised. I would suggest that as the amount of sample available for these tests is rather small, instead of using 75 grams of fat in the official method, half this amount be taken and the quantities of sodium hydroxid, alcohol, water, etc., be reduced proportionately.

Some criticisms have been made against the use of glycerol as a saponification medium since the iodin values of the separated acids as obtained by this method differ from those obtained when alcohol or water is used. I shall greatly appreciate it if those of you who have the time to spare will make a determination of the iodin number of each of the fatty acids after the titer has been determined.

In order to eliminate as far as possible any discrepancies in the reported results of temperature which might arise from differences in standardization of the titer thermometers or of minor variations in methods of stirring and in judging the cooling point, the samples of oils and fats are accompanied by a small sample of fatty acids which has been made here in my laboratory, and I am asking that each of you report the titer temperature of this sample along with the rest of the results.

Cooperative Results on Fats and Oils, 1912.

The results of this collaborative work are given in the following table:

Report of collaborators on methods for making the titer test.

Substance and collaborator.	Methods.			
	Official.	No. 1.	No. 2.	No. 3.
Cocoanut oil:	°C.	°C.	°C.	°C.
L. B. Burnett (Bureau of Chemistry)	23.3	22.0	22.6	23.6
	23.2	22.0	23.6	23.7
		23.6		
		23.8		
F. N. Smalley	23.9	23.6	24.0	23.4
C. P. Long (Globe Soap Co.)	23.1			23.0
R. L. Jickling (Globe Soap Co.)	23.1	23.1	23.2	23.25
R. H. Kerr (Bureau of Animal Industry)	23.4	23.1	22.8	22.4
F. H. Merrill (L. A. Soap Co.)		24.0	24.0	23.7
Maximum	23.9	24.0	24.0	23.7
Minimum	23.1	22.0	22.6	22.4
Average	23.3	23.2	23.4	23.3

Report of collaborators on methods for making the titer test—Continued.

Substance and collaborator.	Methods.			
	Official.	No. 1.	No. 2.	No. 3.
Cottonseed oil:	°C.	°C.	°C.	°C.
L. B. Burnett (Bureau of Chemistry)	36.0	36.8	36.7	36.0
	36.2	36.8	36.7	36.4
	36.2	36.8	36.6	36.5
	.....	36.8	.....	36.4
	.....	36.8	.....	.....
F. N. Smalley	36.1	36.2	36.2	34.7
C. P. Long (Globe Soap Co.)	36.45	.....	.....	36.25
H. L. Jickling (Globe Soap Co.)	36.5	36.7	36.35	36.15
H. B. Esterman (Globe Soap Co.)	36.25	36.3	.....	36.1
R. H. Kerr (Bureau of Animal Industry)	36.5	36.2	36.3	30.0
F. H. Merrill (L. A. Soap Co.)	.....	35.1	.....	31.6
Maximum	36.5	36.8	36.7	36.5
Minimum	36.0	36.0	36.2	30.0
Average	36.3	36.45	36.6	35.0
House grease:				
L. B. Burnett (Bureau of Chemistry)	39.5	39.4	39.3	39.6
	39.6	.....	.....	.....
	39.6	.....	.....	.....
C. P. Long (Globe Soap Co.)	39.3	.....	.....	38.85
R. L. Jickling (Globe Soap Co.)	39.4	39.15	39.25	39.1
H. B. Esterman (Globe Soap Co.)	39.05	39.1	38.6	38.95
R. H. Kerr (Bureau of Animal Industry)	39.4	39.3	39.3	35.6
Maximum	39.6	39.4	39.3	39.6
Minimum	39.05	39.1	38.6	35.6
Average	39.55	39.2	39.1	38.4
Oleo stearin:				
L. B. Burnett (Bureau of Chemistry)	49.9	50.8	50.3	50.1
	50.2	51.3	50.4	50.2
	.....	50.4	.....	.....
	.....	50.45	.....	.....
F. N. Smalley	51.2	51.2	51.4	51.2
C. P. Long (Globe Soap Co.)	51.35	.....	.....	51.3
R. L. Jickling (Globe Soap Co.)	51.3	51.45	51.35	51.35
H. B. Esterman (Globe Soap Co.)	51.4	51.45	51.3	51.25
R. H. Kerr (Bureau of Animal Industry)	51.2	51.3	51.3	46.4
F. H. Merrill (L. A. Soap Co.)	.....	50.6	50.6	50.6
Maximum	51.35	51.45	51.35	51.35
Minimum	49.9	50.6	50.3	50.1
Average	50.9	51.0	51.0	50.5
Tallow:				
L. B. Burnett (Bureau of Chemistry)	42.8	42.7	42.7	42.5
	42.7	.....	42.4	42.8
C. P. Long (Globe Soap Co.)	42.75	.....	.....	42.7
R. L. Jickling (Globe Soap Co.)	42.85	42.8	42.7	42.8
H. B. Esterman (Globe Soap Co.)	42.75	42.7	42.7	42.65
R. H. Kerr (Bureau of Animal Industry)	43.0	43.0	42.8	41.6
Maximum	43.0	43.0	42.8	42.8
Minimum	42.7	42.7	42.4	41.6
Average	42.8	42.8	42.6	42.5

Comments of Collaborators.

F. H. Merrill: The great discrepancy on the cottonseed oil has much interested me, and we have gone over this ground repeatedly to make sure that the low results with Methods 2 and 3 were due to no faulty manipulation. I am at a loss to explain such results, from the fact that they duplicate themselves regularly on repeated tests.

C. P. Long: The use of flasks for Methods 1 and 2 seems hardly advisable as we experienced much trouble from foaming, which can be obviated by the use of a large beaker such as we used in most of our work with these methods.

The addition of water, especially hot water, to a very hot glycerol solution seems to have an element of danger entirely overcome in Method 3 by pouring the glycerol into the water. On the whole, we believe that the method of saponification does not give rise to as much variation in titers as other factors, such as method of stirring of different operators, the amount of supercooling, the variation in room temperature, and the amount of fat used.

F. N. Smalley mentioned the danger of burning the fatty acids in Method 3, a difficulty experienced in our own laboratory, and said: We find that with Method 3 it is extremely hard to avoid burning the fatty acids, and we feel that this is the cause of the low value, especially of the cottonseed oil. We can not see any particular advantage gained in either of the three proposed methods over the official method and trust

that the general consensus of opinion agrees with us and that the official method will remain as it now stands.

R. H. Kerr: Methods 1 and 2 are almost if not quite as trustworthy as the official method and far more rapid. There is but little choice between them, although I prefer No. 1. Method 3 is worthless.

As a result of the suggestions of the collaborators and the experience of Mr. Burnett and myself, the following method, which apparently combines the best points of all the others and eliminates so far as possible their shortcomings, has been worked out:

New Method.

Heat to 150° C. in an 800 cc beaker 75 cc of a glycerol potassium hydroxid solution, made by dissolving 25 grams of potassium hydroxid in 100 cc of high-test glycerin; then add 50 cc of the oil or melted fat, previously filtered, if necessary, to remove foreign substances. Saponification in many cases takes place almost immediately, but the heating, with frequent stirring, should be continued for 15 minutes, care being taken that the temperature does not rise much above 150° C. When the saponification is complete, as indicated by the perfectly homogeneous solution, pour the soap into an 800 cc casserole containing about 500 cc of nearly boiling water, carefully add 50 cc of 30 per cent sulphuric acid, and heat the solution, with frequent stirring, until the layer of fatty acids separates out perfectly clear. Transfer the fatty acids to a tall separatory funnel, wash three or four times with boiling water to remove all mineral acids, draw them off into a small beaker, and allow to stand on a steam bath until the water has settled out and they are clear. Filter into a dry beaker and heat to 150° C. on a thin asbestos plate, stirring continually with the thermometer; transfer to a titer tube, filling it to within 1 inch of the top, and take the titer as indicated in the present provisional method.[1]

Discussion.

Only a few of the collaborators reported the iodin numbers upon their fatty acids, but, judging from the figures received and the work of Mr. Burnett, it seems probable that the difference between the iodin values of the fatty acids obtained by the official and proposed methods will vary much more widely than the titer and that this variation is due to some extent to the difficulty in entirely washing out the mineral acid used in the decomposition and to the time and manner of drying. The effect of these factors upon the titer was investigated thoroughly in 1904[2] in the cases of oleostearin, edible tallow, yellow grease, and cottonseed oil, but might be further studied in connection with the titer of cocoanut oil and similar fats, having a high volatile acid content, and the drying oils.

The associate referee wishes to acknowledge his indebtedness to his collaborators, who kindly furnished the necessary material for the samples submitted.

Recommendations.

It is recommended—

(1) That the Emery method (U. S. Dept. Agr., Bureau of Animal Industry Cir. 132) reported at the 1911 meeting for the detection of added beef fat in lard be made provisional.

(2) That final action be taken to make the provisional method for Preparation of Sample (Bul. 107, Rev., p. 129) official.

(3) That method (*c*) Zeiss Butyro-Refractometer (Bul. 107, Rev., p. 132) be finally adopted as official.

(4) That Method 12, Free Fatty Acids (Bul. 107, Rev., p. 142) be finally adopted as official.

(5) That the Halphen reaction for cottonseed oil (Bul. 107, Rev., p. 144, 17 (*b*)) be finally adopted as official.

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[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 107, Revised, p. 135.

[2] U. S. Dept. Agr., Bureau of Chemistry Cir. 22.

(6) That the Baudouin test for sesame oil (Bul. 107, Rev., p. 146, 17 (*e*)) be finally adopted as official.

(7) That the Villavecchia test for sesame oil (Bul. 107, Rev., p. 146, 17 (*f*)) be finally adopted as official.

(8) That any cut appearing in the text of the chapter on fats and oils, Bulletin 107, Revised, be considered merely as an illustration and not as an integral part of the method.

(9) That 75 degrees in addition to or instead of 100 degrees be adopted provisionally with a view to ultimately making it official for the determination of the specific gravity of high melting point fats.

This would not be a substitution but would be a new method in that sense, and its adoption is recommended, which would be on first reading this year.

(10) That the glycerol method for the preparation of fatty acids for use in the titer test, as reported in detail in the report of the 1912 associate referee on fats and oils, be adopted as a provisional method.

REPORT ON DAIRY PRODUCTS.

By A. E. Paul, *Associate Referee.*[1]

At the last meeting the writer proposed a method of extracting fat from milk, cream, ice cream, evaporated milk, and sweetened condensed milk in quantity sufficient to permit of examination for the presence of foreign fat. This was recommended for study during the present year.

In working with the method it was found that in the case of abnormally rich cream, especially if it contains foreign fat, the precipitate produced by the copper reagent was insufficient in quantity to entangle all the fat, so that in washing the precipitate more or less fat passed through the filter. Accordingly a modification was devised which obviates this difficulty.

Cooperative Work on Dairy Products, 1912.

Samples of various kinds of dairy products containing varying proportions of foreign fat were submitted to the collaborators, together with both the proposed method and the modification. It was requested that the reports include the following determinations:

Fat by the Roese-Gottlieb method.
Fat recovered by the proposed method.
Reichert-Meissl value of the fat.
Refractive index of the fat.
Valenta test.
Approximate composition of the fat.

[1] Presented by A. S. Mitchell.

Reports by collaborators on dairy products.

Sample and collaborator.	Fat by Roese-Gottlieb method.	Fat recovered by proposed method.	Reichert-Meissl value.	Refractive index at 25° C.	Valenta test.	Approximate composition of fat.
1. Milk—3 per cent butter fat, 1 per cent oleo (fat present contains 25 per cent oleo):	*Per cent.*	*Per cent.*			° *C.*	
A. S. Wells	3.69 3.68	3.53	18.6	1.4595	.....	About half foreign fat.
E. H. Berry	3.97 3.96	3.54 3.57	19.7 21.5	1.4590	37	About 25 per cent foreign fat.
J. T. Keister	3.88 3.91	3.45	22.95	1.4614	29	15 to 20 per cent oleo.
2. Cream—5 per cent butter fat, 15 per cent oleo (fat present contains 75 per cent oleo):						
A. S. Wells	23.37 23.51	23.50	10.05	1.4618	.....	Sample lumpy.
E. H. Berry	20.54 20.59	20.22 20.25	8.5 8.7	1.4622	59	About 75 per cent foreign fat.
J. T. Keister	.....	.....	8.87	1.4621	56	70 per cent oleo.
3. Unsweetened evaporated milk—no oleo:						
Geo. B. Taylor	8.23 8.13 8.01	.....	27.2	1.4599	66(?)	Pure butter fat.
A. S. Wells	7.68 7.83	7.82	27.5	1.4592	.....	Do.
E. H. Berry	8.34 8.47	7.82 8.02	28.3 28.3	1.4595	33	Do.
G. G. Parkin [1]	7.94 8.08	7.98 8.08	.....	.....	.....	
J. T. Keister	8.266 8.234	8.15	28.4	1.4596	28	Do.
4. Sweetened condensed milk—1 per cent butter fat, 11 per cent oleo (fat present contains 91.7 per cent oleo):						
Geo. B. Taylor	11.27 11.32 11.35	.....	4.18	1.4636	Above 100	Foreign fat, 90 per cent.
A. S. Wells	11.41 11.22	11.39	3.1	1.4613	.....	Very little or no butter fat.
E. H. Berry	11.56 11.45	11.95 11.54	3.9	1.4623	81	Practically no butter fat.
J. T. Keister	11.68 11.65	11.18	3.27	1.4630	65	90 per cent oleo.
5. Ice cream—10 per cent butter fat, 10 per cent oleo (fat present contains 50 per cent oleo):						
Geo. B. Taylor	[2] 15.8	.....	17.7	1.4625	.....	Suspicions of foreign fat.
A. S. Wells	18.04 16.08	19.32	12.15	1.4613	.....	About half butter fat.
E. H. Berry	20.15 20.14	19.94 20.07 20.24	13.7 13.5	1.4618	62 63	40 to 50 per cent butter fat.
J. T. Keister	20.45 20.54	19.95	14.80	1.4624	41	48 per cent oleo.

[1] Fat by Harding-Parkin method, 8.27 per cent (method similar to Werner-Schmidt, but using acetic acid, CCl_4, and petroleum ether, to appear in J. Ind. Eng. Chem.).
[2] Badly separated.

Discussion of Results.

The nature of the products must be taken into account in considering the above results. The discrepancies in the Roese-Gottlieb results are undoubtedly due entirely to the separation of the fat in transit.

It appears from the results that the Reichert-Meissl value is the constant upon which dependence must be placed regarding the approximate percentage of foreign fat. Mr. Keister's and Mr. Berry's quantitative figures are based entirely on this constant. The Valenta test is easily applied and may be useful as a confirmatory determination. Mr. Keister stated that he considered the Valenta number of very little value. Nevertheless, his results and Mr. Berry's, based on this test, are quite indicative in cases where 50 per cent or more of adulterant is present. The same may be said with reference to the refractive index.

Regarding the method of extraction, it is unfortunate that not all collaborators stated whether they considered the modification necessary or desirable. Mr. Keister stated that "in case of cream samples the difficulty of part of the fat passing through the filter was experienced. The modified method, however, was satisfactory, although the filtering was somewhat slow." Mr. Berry stated that "the method as originally proposed works well with all but samples containing very much fat, especially if foreign fat is present. In the case of ice cream there was a slight tendency for the fat to filter through, but the sample contains more fat than commercial ice cream does, and ordinarily the method will work well. On the cream sample the original method was not entirely satisfactory, as the fat filtered through. The modification proposed worked very well on this sample and on others on which it was tried."

Recommendations.

The following recommendations are respectfully submitted: (1) That the original method as repeated below be adopted as provisional for milk, evaporated milk, sweetened condensed milk, ice cream, and thin cream:

Into a 1,000 cc beaker weigh 100 grams (milk 250 grams) of the material. Add 300 cc of water, mix thoroughly, and bring to a boil. Now add, while boiling, very gradually, 25 cc of Soxhlet's copper sulphate solution diluted with 100 cc of water.

In a Büchner funnel, wet a filter of suitable size and of loose texture. Filter with suction, and wash three times with a little boiling water; filter as dry as possible. Remove the cake, which should be dry enough to be broken up easily between the fingers; break into small particles, and dry in the open air overnight. Grind in a mortar with sufficient anhydrous copper sulphate (usually 25 grams is enough) and let stand for a few minutes, or until the product seems quite dry and not at all lumpy.

Into the inner tube of a large Johnson or other extractor place a layer of the anhydrous copper sulphate and then the powdered mixture. Place a loose plug of cotton on top of the mixture, and extract with ordinary ether. The ether should be poured into the extractor and allowed to percolate through before the heating is begun. Approximately 50 cc of the solvent will be required. Dry and weigh the fat.

(2) That the following modification for rich cream be further studied:

Add 50 grams of the material to 25 cc of boiling Soxhlet copper solution in a 250 cc beaker, stirring briskly. Cover the filter with a thin layer of fibrous asbestos mixture, carefully covering the sides as far up as possible. Filter, wash once or twice with cold water, and proceed as above.

A paper on "The modified Babcock test for fat in sweetened dairy products," by J. O. Halverson, of the University of Missouri, was presented by Mr. Trowbridge and later printed in the Journal of Industrial and Engineering Chemistry, 1913, volume 5, pages 403–409.

REPORT ON CEREAL PRODUCTS.

By H. L. White, *Associate Referee.*

The work outlined for the year included a study of methods for the estimation in wheat flour of soluble carbohydrates, gluten, gliadin, edestin, and leucosin, amid nitrogen, nitrous nitrogen, moisture, and acidity of water extract. Three samples of flour were furnished each collaborator: Sample A from hard wheat, Sample B from durum wheat, and Sample C, which was bleached with nitrogen tetroxid. Five chemists offered to collaborate in this work but only three sent in results. Owing to the small number of data obtained, the associate referee has not deemed it advisable to present many recommendations for action at this meeting.

Methods and Results.

SOLUBLE CARBOHYDRATES AS DEXTROSE.

The alcohol extraction method of Bryan, Given, and Straughn (Bureau of Chemistry Cir. 71) was subjected to further trial with the following results:

Soluble carbohydrates as dextrose.

Analyst.	Sample A.	Sample B.
	Per cent.	*Per cent.*
R. F. Beard, Agricultural College, N. Dak	[1] 1.24	[1] 1.99
B. R. Jacobs, Washington, D. C	1.02	1.59
	.95	1.567

[1] Average of 5 determinations.

Last year this method gave close results in the hands of four analysts, but no explanation can be given for this marked difference in results. Sample B is made from the same wheat as a sample used in last year's collaborative work, in which 1.83 per cent soluble carbohydrates was found.

Miss Leila Dunton, Manhattan, Kans., said of her work this year: "The alcohol-digestion method for the determination of dextrose I have found very unsatisfactory. After so long a digestion at that temperature I found the solutions very hard to filter, and even with the most careful covering to prevent loss by evaporation the loss is sufficient to affect the results materially. We are at present using in this laboratory the extraction with a 0.2 per cent solution of sodium carbonate and find it very satisfactory."

GLUTEN.

Method I (Bureau of Chemistry Bul. 122, p. 54): Dough up 30 grams of flour with 18 cc of water conveniently in an 8-ounce mortar. Weigh off 16 grams of dough, equivalent to 10 grams of flour. Place in water at room temperature for one hour and carefully wash out the starch over bolting cloth or a fine horsehair sieve. After expressing all globules of water, weigh the moist gluten upon a watch glass. Dry in a desiccator for 24 hours and complete drying in water oven.

Method II (modification of Method I, suggested by G. A. Olson): Dough up 10 grams of flour with 6 cc of water. After weighing the wet gluten, place in a vacuum oven and dry for three hours under 65 cm vacuum at 85° C. The reduced pressure and the temperature combine to cause the gluten to expand rapidly.

Comparative results obtained by different methods.

Analyst.	Sample A.				Sample B.			
	Method I.		Method II.		Method I.		Method II.	
	Wet.	Dry.	Wet.	Dry.	Wet.	Dry.	Wet.	Dry.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
G. A. Olson, Pullman, Wash	36.8	14.30	37.5	12.99	42.9	16.89	43.9	15.24
	35.9	14.26	37.0	12.60	44.5	17.47	44.4	15.41
B. R. Jacobs, Washington, D. C	42.0	14.3	.....	.....	49.7	16.0	.....	.....
Leila Dunton, Manhattan, Kans	45.45	15.47	.....	.....	36.7	12.07	.....	.....

These results seem to indicate that by Method II more water can be removed from the gluten.

GLIADIN.

Method I (suggested by Ralph Hoagland in J. Ind. Eng. Chem., 1911, *3* (2): 840): In this method 50 per cent alcohol (by weight) is used.

GLIADIN NITROGEN.

Results obtained by use of alcohol of different strengths.

Analyst.	Sample A.		Sample B.	
	Method I.	Use of 70 per cent alcohol (by volume).	Method I.	Use of 70 per cent alcohol (by volume).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
R. F. Beard, Agricultural College, North Dakota	1.27	.....	1.60	.....
B. R. Jacobs, Washington, D. C	1.40	1.25	1.87	1.61
G. A. Olson, Pullman, Wash	.....	1.21	.....	1.55
Leila Dunton, Manhattan, Kans	1.28	.....	1.66	.....

The wide variation in these results indicates the necessity for further trial of the two methods.

EDESTIN AND LEUCOSIN, AND AMID NITROGEN.

The method suggested for these determinations is the one given in Bulletin 122, page 54, using the factor 5.7.

Total salt soluble nitrogen, edestin and leucosin nitrogen, and amid nitrogen.

Analyst.	Sample A.			Sample B.		
	Total nitrogen.	Edestin and leucosin.	Amid nitrogen.	Total nitrogen.	Edestin and leucosin.	Amid nitrogen.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
G. A. Olson, Pullman, Wash.	0.51 .50 .50 .56	0.41	.....	0.50 .52 .47 .52	0.427	.....
Leila Dunton, Manhattan, Kans.	.....	.78	.25	.....	.84	.25
B. R. Jacobs, Washington, D. C.	.533 .547	....	.056 .056	.589 .589	....	.070 .070
Average.	.525	.....	.....	.531	.....	.....

From the results thus far obtained this method appears to be fairly satisfactory. It is desirable, however, that a larger number of analysts should give it a trial.

ACIDITY OF WATER-EXTRACT.

At the meeting of the association in 1911 the following recommendation was adopted: That methods for the determination of the acidity of the water extract of flour be further studied with reference to the temperature of the water and time of extraction. In following out this recommendation the following variations of time and temperature are suggested: Time, 2 hours, 4 hours, 6 hours. Temperature, 40° C., 60° C.

Method I: Weigh 18 grams of flour into a 500 cc Erlenmeyer flask and add 200 cc of distilled water free from carbon dioxid. Place flask in a water oven kept at temperature of experiment, and for time indicated, shaking vigorously every half-hour. Filter through dry double filters, reject the first 10 cc of filtrate, then continue filtering until 100 cc are obtained. Titrate with twentieth-normal sodium hydroxid, using carefully neutralized phenolphthalein as an indicator. Each cubic centimeter of twentieth-normal sodium hydroxid solution represents 0.05 per cent of acidity as lactic acid.

Acidity of water-extract results obtained by different temperatures and for different extraction periods.

[Expressed as per cent lactic acid.]

Sample and analyst.	At 40° C.			At 60° C.		
	2 hours.	4 hours.	6 hours.	2 hours.	4 hours.	6 hours.
Sample A:						
R. F. Beard, Agricultural College, N. Dak.	*Per cent.* 0.223	*Per cent.* 0.248	*Per cent.* 0.277	*Per cent.* 0.260	*Per cent.* 0.292	*Per cent.* 0.283
Leila Dunton, Manhattan, Kans.	.245	.260	.271	.290	.290	.325
B. R. Jacobs, Washington, D. C.	.180	.210	.310	.170	.180	.170
Sample B:						
R. F. Beard, Agricultural College, N. Dak.	.209	.245	.268	.256	.277	.293
Leila Dunton, Manhattan, Kans.	.288	.320	.355	.350	.372	.400
B. R. Jacobs, Washington, D. C.	.190	.240	.280	.190	.220	.200

R. F. Beard: Was unable at any time to obtain 100 cc of filtrate from flour extract at 60° C. for six hours. In several cases the same was true of flour at 60° C. for four hours. It was also impossible to get clear solutions at 60° C. In general, found that flour extracts at 60° C., even for two hours only, were quite difficult and unsatisfactory to manipulate.

B. R. Jacobs: In the directions for making the determination of acidity of the water-soluble extract, the quantity of phenolphthalein indicator to be used is not given, although the point at which the end point is reached is very materially affected by the quantity of indicator. In this laboratory 2 cc of indicator, prepared in the regular way as given on page 138 of Bulletin 107, Revised, Bureau of Chemistry, United States Department of Agriculture, were used. Most other determinations of acidity require only a few drops of phenolphthalein indicator, but in the case of flour the end point appears to be very much more definite and sharp when a large quantity is used.

These results indicate that extraction for two hours at 40° C. is too short a period to remove all of the acid-reacting material from the flour, nor is it certain from these determinations that six hours' extraction at 40° C. is enough. The possibility of the gradual formation of acid-reacting material at 40° C. must be considered. The results obtained at 60° C. would seem to indicate that in general the maximum amount of acid-reacting material is extracted in four hours. The solutions at this temperature, however, are unsatisfactory to work with. It is recommended that the next referee give this subject his immediate attention.

MOISTURE.

Relative to the determination of moisture in wheat flour the association at its meeting in 1911 adopted the recommendation "that a further study be made of the efficiency of the vacuum desiccator as compared with the vacuum oven." The associate referee suggested that each collaborator make a series of determinations of moisture on Samples A and B, using both desiccator and oven.

Moisture obtained under varying conditions of temperature and pressure.

Analyst.	Sample A.			Sample B.		
	At 100° C. in vacuum.	At 85° C. and 60 mm.	72 hours in vacuum desiccator.	At 100° C. in vacuum.	At 85° C. and 60 mm.	72 hours in vacuum desiccator.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
B. R. Jacobs, Washington, D. C.	11.61	.....	.....	11.23	.....	.....
	11.65	.....	.....	11.21	.....	.....
G. A. Olson, Pullman, Wash.	.....	11.55	6.14	.....	11.13	5.75
	.....	11.48	6.02	.....	11.08	5.59

REPORT ON CONDIMENTS OTHER THAN SPICES.

By W. J. McGee, *Associate Referee.*

The work of the last year has been entirely on tomato ketchup. In accordance with the recommendations of the preceding year, two samples of ketchup were prepared, one of sound tomato pulp (A) and the other of tomato pulp which had been allowed to stand in porcelain dishes in the laboratory until completely filled with mold (B), using the same formula for each. Samples of each were sent to those who had volunteered to do some work on this subject, with the request that the following determinations be made:

STATEMENT OF METHODS.

Total solids.—Determine as usual, using from 3 to 5 grams and drying at the temperature of boiling water for 4 hours. The sample should be spread out in a thin layer for drying.

Insoluble solids.—Wash 20 grams repeatedly with hot water, centrifuging after each addition of water. Pour the clear supernatant liquid through a tared double filter on a Büchner funnel. A cylinder 1 to 1¼ inches in diameter and 5 to 6 inches long is convenient for washing and centrifuging. This may be prepared by shortening a colorimeter tube. After 4 or 5 washings, transfer the remaining insoluble matter to the filter and finally dry for 2 hours at 100° C. The filter paper used in this determination should be dried for 2 hours at 100° before the original weighing.

Soluble solids.—Calculated by difference.

Ash.—Determine in the usual manner. Avoid heating above dull redness.

Alkalinity of ash.—Transfer the ash to a 100 cc flask and dilute to the mark. This solution, together with a small amount of insoluble matter, is shaken thoroughly and an aliquot portion pipetted off as quickly as possible for the determination of alkalinity of the ash. Determine as under "Alkalinity of insoluble ash," Bulletin 107, Revised, page 69, and report as cubic centimeters of tenth-normal acid per ash of 1 gram of ketchup. Also report as per cent potassium carbonate in the salt-free ash.

Sodium chlorid.—Determine on an aliquot portion of the ash solution by Mohr's method.

Reducing sugars before inversion.—Weigh 10 grams into a 100 cc flask, clarify with an excess of normal lead acetate, dilute to the mark, and filter. Remove the excess of lead with dry sodium sulphate or sodium carbonate, filter, and determine reducing sugars by the Munson and Walker method, Bulletin 107, Revised, page 242. Calculate the sugars as per cent invert sugar and sucrose. (See table in Bulletin 107, Revised, pp. 243 to 251, column 5.)

Reducing sugars after inversion.—Transfer 50 cc of the filtrate obtained in the previous determination to a 100 cc flask, add 5 cc concentrated hydrochloric acid, let stand overnight, neutralize exactly with sodium hydroxid, cool, make to the mark, and determine reducing sugars as before. Calculate as invert sugar.

Sucrose.—Calculate as directed on pages 41 and 42 of Bulletin 107, Revised.

Polarization after inversion.—Determine in the usual manner. Use the normal weight of ketchup and allow to stand overnight at room temperature for inversion. Report as polarization on the normal weight—that is, 26 grams in 100 cc.

Total acids, as citric.—Use 5 grams and determine as under "Vinegar," Bulletin 107, Revised, page 103. One cubic centimeter of tenth-normal alkali equals 0.0063 gram of citric acid.

Volatile acids, as acetic.—Determine volatile acids in the usual manner, using 25 grams of sample. One cubic centimeter of tenth-normal alkali equals 0.006 gram of acetic acid. Reserve the neutralized distillate for the determination of butyric and formic acids.

Butyric acid.—Evaporate to dryness the neutralized distillate obtained under "Volatile acids" in a weighed platinum dish on the steam bath. Heat for 2 hours in an oven at 100° C. and weigh. From the alkali used and the weight of the salts calculate the per cent of sodium in the salts. Decompose the salts with about 5 cc of 10 per cent sulphuric acid. The acid residue thus obtained should be smelled to ascertain the presence of butyric acid. The sodium content of sodium acetate is 28 per cent, while that of sodium butyrate is 20.5. Owing to the natural limits of error, a lower sodium content than 28 per cent may occur, even in the absence of butyric acid; but if the amount of sodium present in the salt is found by the calculation directed above to be as low as 27 per cent, it is our experience that a considerable amount of butyric acid is present. A much smaller amount can be detected by smell, however, and this method of calculation is only of value in determining the approximate amount of butyric acid where considerable quantities are present.

Formic acid.—To the salt residue acidified with sulphuric acid obtained in the preceding determination small quantities of magnesium are added, the action of the magnesium upon the acid being allowed to continue for about an hour. Formic acid present is thus reduced to formaldehyde, which may be detected by the Hehner or Leach method. If the reaction is positive, the presence or absence of formaldehyde in the original sample should be demonstrated.

Fixed acids, as citric.—Calculate by difference.

Lactic acid.—Make 100 grams of ketchup up to 500 cc with water, mix thoroughly, centrifuge; pour off 400 cc through a filter, add 10 cc of 20 per cent lead acetate solution, and make up to 500 cc. After centrifuging again, 400 cc should be poured off through a filter and a slight excess of dilute sulphuric acid added. Settle precipitate

with centrifuge and pour directly into evaporating dish. Wash the lead sulphate precipitate at once with about 50 cc of water and filter into the evaporating dish. Evaporate on steam bath to volume desired for the extraction apparatus. Extract for 18 to 20 hours in a liquid extractor with washed ether. (Ether sufficiently pure for this purpose may be prepared by shaking out ordinary ether once with sodium hydrate and then 10 times with small quantities of water.) To the ether extract is added about 20 cc of water, and the ether is evaporated to dryness, taken up with water, and filtered. Care must be taken to remove all traces of ether. The water is added before evaporation because of the possible presence of traces of sulphuric acid, which might otherwise char the lactic acid. Approximately 3 grams of sodium hydrate are then added to the water solution, and 50 cc of a 1.5 per cent solution of potassium permanganate are added from a pipette. This mixture is heated on a water bath at 100° C. for one-half hour. At the end of that time, or before, if the color is not a decided blue-black or purple, but is green or colorless above the layer of brown precipitate, more standard permanganate in measured portions is added until, after heating one-half hour on a boiling water bath, the color is a blue-black or purple. The oxidation is then complete The hot solution is strongly acidified with dilute sulphuric acid, about 50 cc of 10 per cent sulphuric acid, and standard oxalic acid run in from a burette until the solution is decolorized. (In this laboratory a 5 per cent solution of oxalic acid is used for that purpose.) Any slight excess of oxalic acid is titrated back with the same standard permanganate solution. It should be understood that any standard permanganate and oxalic acid solutions may be used within reasonable limits of strength. In alkaline solution, the permanganate oxidizes the lactic acid quantitatively to oxalic acid according to the equation

$$2\ C_3H_6O_3+10\ KMnO_4=2\ (COOH)_2+4H_2O+2\ CO_2+5\ MnO_2+5K_2MnO_4.$$

Then, in acid solution, the oxalic acid is further oxidized by the permanganate to carbon dioxid and water according to the equation

$$5\ (COOH)_2+2KMnO_4+3H_2SO_4=10\ CO_2+8H_2O+K_2SO_4+2MnSO_4.$$

Calculation: The total weight of permanganate used in the oxidation of the lactic acid is determined by subtracting the permanganate equivalent of the oxalic acid used from the total amount used. The weight of permanganate times 0.237 equals the weight of lactic acid.

Citric acid.—Weigh 25 grams into a 250 cc beaker, make up to approximately 200 cc with 95 per cent alcohol; allow to stand with frequent stirring for four hours; filter through a folded filter, and wash with 50 cc of 80 per cent alcohol. To the filtrate add 10 cc of 20 per cent barium acetate solution, stir well with a glass rod, and allow to stand overnight. In the morning filter on a Gooch crucible, washing with 50 per cent alcohol; dry two hours in an oven at 100° C., and weigh. Weight of precipitate×0.49=citric acid.

Pectin determination.—Filter the ketchup through a folded filter and weigh 25 grams of the filtrate into a 250 cc beaker. Add 95 per cent alcohol to a total volume of 200 cc. Mix thoroughly; allow to stand overnight. The so-called pectin bodies will probably be found firmly sticking to the sides and bottom of the beaker. If this is the case, pour off the alcohol, dissolve the pectins in a small amount of warm water, transfer to a large platinum dish, make up to approximately 100 cc with 95 per cent alcohol, and allow to stand for 12 hours. Pour off the alcohol, dry the pectins contained in the dish for two hours in an oven at 100° C., and weigh. Ash at a dull red heat, weigh, and subtract the weight of the ash. The loss on ignition represents the so-called pectin bodies for a 25-gram sample.

Sand.—Weigh 100 grams into a 2 or 3 liter beaker, nearly fill the beaker with water, and mix the contents thoroughly. Allow to stand five minutes and decant the supernatant liquid into a second beaker; refill the first with water and again mix the contents. After five minutes more decant the second beaker into the third, the first into the second, refill, and again mix the first. Continue this operation, decanting from the third beaker into the sink until the lighter material is washed out from the ketchup; then collect the sand from the three beakers in a tared Gooch crucible. Dry, ignite, and weigh. Attention is especially called to the fact that under "Sand" should only be reported the figure obtained by this method. The results obtained by the determination of ash insoluble in hydrochloric acid are not applicable to the determination of sand in tomato ketchup, partly because the percentage present is so small and the sand is so unevenly distributed that reliable results can only be obtained by taking a larger sample than is possible in the determination of ash.

Qualitative determinations.—Make qualitative examinations for benzoic acid, saccharin, boric acid, starch.

Report also the following: Sugar-free, salt-free soluble solids; ratio of insoluble solids to sugar-free, salt-free soluble solids; ratio of lactic acid to citric acid; ratio of citric acid to sugar-free, salt-free soluble solids.

Examine the original sample before mixing and report the presence and appearance of any sediment which may appear on the bottom.

Analytical Results.

The following analyses were made:

Comparative percentage results by three analysts on two samples of ketchup.

Determinations.	"A" samples (sound tomatoes).			"B" samples (decayed tomatoes).		
	L. C. Johnson.	E. H. Grant.	W. J. McGee.	L. C. Johnson.	E. H. Grant.	W. J. McGee.
Total solids	19.58	20.40	20.00	21.16	22.60	21.80
Insoluble solids	1.21	.82	.56	1.86	1.01	.95
Soluble solids	18.37	19.60	19.44	19.30	21.60	20.84
Ash	3.64	3.89	3.69	4.26	4.53	4.34
Alkalinity of ash (cc tenth-normal acid per 1 gram of ketchup)	.80	.62	.60	1.20	.80	.69
Sodium chlorid	2.95	2.94	2.99	3.57	3.38	3.44
Reducing sugars before inversion	12.80	13.15	12.79	10.14	10.81	11.00
Reducing sugars after inversion	13.12	13.30	13.10	13.28	13.71	13.25
Sucrose	.30	.14	.29	3.00	2.76	2.14
Polarization after inversion (°V)	27° C. / −4.15	32° C. / −2.8	32° C. / −4.0	27° C. / −3.10	32° C. / −3.7	32° C. / −4.26
Total acids as citric	1.32	1.45	1.48	1.64	1.69	1.73
Volatile acids as acetic	.58	.68	.58	.76	.76	.71
Fixed acids as citric	.74	.72	.87	.88	.94	1.04
Butyric acid	Present.	None.	None.	Present.	None.	None.
Formic acid	Trace.	None.	None.	Trace.	None.	None.
Lactic acid	.43 / .35	.41 / .39	.39 / .27	.85 / .73	.45 / .39	.69 / .56
Citric acid	.59 / .64	.57	.57	.48 / .50	.53	.67
Pectins	.53	.65	.47	.67	.78	.40
Sand	.005			.014		
Sugar-free, salt-free soluble solids	2.32	3.4	3.37	2.59	5.4	4.26
Ratio insoluble solids to sugar-free, salt-free soluble solids	1:1.9	1:4.1	1:6.0	1:1.4	1:5.4	1:4.48
Ratio lactic to citric acid	1:1.6	1:1.45	1:1.73	1:0.62	1:1.35	1:1.06
Ratio citric acid to sugar-free, salt-free soluble solids	1:3.7	1:6.0	1:5.91	1:5.3	1:10.0	1:6.30

Other Work on Ketchup.

The second recommendation of last year was that some experiments be made to ascertain to what extent the nitrogen ratios can be influenced by the spice content of ketchups. Owing to the press of other work this line of investigation had to be abandoned.

The recommendation that the number of determinations be reduced, if possible, was carried out, and no nitrogen determinations were asked of the collaborators this year.

It was thought that some substances having high ether extracts might, upon putting them through the lactic acid method, yield something that would be oxidized by the permanganate of potash and be reported as lactic acid. In this connection E. H. Grant, of the New Orleans laboratory, made some experiments with the materials commonly used in ketchup. The method was as follows:

Boil 50 grams each of mustard, allspice, and black pepper for half an hour with 500 cc of 0.5 per cent acetic acid, keeping the volume at the mark by the addition of hot water. After cooling, treat the mixtures by the lactic acid method precisely as if they were ketchup. The lactic acid in the vinegar was also determined.

The results are as follows, reporting the oxidizable extract from the spices, whatever it was, as lactic acid:

		Lactic acid.
Vinegar	per cent..	0.07
Mustard	do....	1.35
Allspice	do....	.77
Black pepper	do....	.424

Multiplying these results by the percentage of each used in the ketchup, we find that about 0.05 per cent of the apparent lactic acid of the ketchup is furnished by the spices and vinegar, and that more than half of this is real lactic acid derived from the vinegar.

From these results, therefore, it may be considered that substances high in ether extract, such as spices, do not interfere materially with the determination of lactic acid in ketchup.

It is recommended that the determination of lactic acid by the method given below be made provisional, and that all the other methods below, as they are but modifications of those already official, be made official for ketchup.

METHODS FOR THE ANALYSIS OF TOMATO PRODUCTS.

Determination of total solids.—Weigh 10 grams into a platinum dish having a diameter of about 6 cm, spread out the sample in a thin layer, and dry at the temperature of boiling water for 4 hours.

Determination of insoluble solids.—Wash 20 grams repeatedly with hot water, centrifuging after each addition of water and pouring the clear supernatant liquid through a tared triple filter paper on a Büchner funnel. After 4 or 5 washings transfer the remaining insoluble matter to the filter, dry for 2 hours at 100° C., cool in a desiccator, and weigh rapidly in the air. A triple filter paper is used, as strong suction will usually rupture a single or double layer of paper. The paper used should be dried for 2 hours at 100° C., cooled in a desiccator, and the original weighing made rapidly in the air. A cylinder 1 to 1¼ inches in diameter and 5 to 6 inches long is convenient for washing and centrifuging. This may be prepared by shortening a colorimeter tube.

Determination of soluble solids.—Calculate by difference.

Determination of ash.—Evaporate and ignite 10 grams as described under "Fruits and Fruit Products," Bureau of Chemistry Bulletin 107, Revised, page 78, 5–a.

Determination of alkalinity of ash.—Follow the method described under "Fruits and Fruit Products," Bulletin 107, Revised, page 78, 5–b–1. A standard hydrochloric acid may be used instead of nitric if it is not desired to determine chlorin in solution.

Determination of sodium chlorid.—Proceed as directed under "Fruits and Fruit Products," Bulletin 107, Revised, page 79, 5–b–2, using either an aliquot of the solution obtained in the determination of the alkalinity of the ash or the whole portion ashed.

Determination of reducing sugars before inversion.—Weigh 10 grams into a 100 cc flask, clarify with a slight excess of normal lead acetate solution, dilute to the mark, and filter. Remove the excess of lead with dry sodium or potassium oxalate or sodium carbonate. Filter and determine reducing sugars by the Munson and Walker method, Bulletin 107, Revised, page 242. Calculate the sugars as per cent invert sugar and sucrose (see table, Bulletin 107, Revised, pp. 243 to 251, column 5).

Determination of reducing sugars after inversion.—Transfer 50 cc of the filtrate obtained in the previous determination to a 100 cc flask. If sodium carbonate was used to precipitate lead, add acetic acid to acid reaction. Now add 5 cc of concentrated hydrochloric acid, let stand overnight, nearly neutralize with sodium hydroxid, and finish the neutralization with sodium carbonate. Cool, dilute to the mark, and determine reducing sugars as before. Calculate as invert sugar.

Determination of sucrose.—Calculate as directed on pages 41 and 42 of Bulletin 107, Revised.

Determination of polarization after inversion.—Use normal weight of sample and prepare the solution as directed under reducing sugars before and after inversion.

Determination of total acids as citric.—Use 5 grams of the sample and determine as under "Vinegar," Bulletin 107, Revised, page 103, 9. One cubic centimeter N/10 alkali=0.0064 gram of anhydrous citric acid.

Determination of volatile acids as acetic.—Follow the method described under "Vinegar," Bureau of Chemistry Bulletin 107, page 103, 10, using 25 grams of sample. One cubic centimeter N/10 alkali=0.006 gram acetic acid. Reserve the neutralized distillate for detection of butyric acid.

Detection of butyric acid.—Evaporate the neutralized distillate obtained under "Volatile acids" to dryness on the steam bath. Decompose the residue with about 5 cc of 10 per cent sulphuric acid and note whether the odor of butyric acid is present.

Determination of fixed acids as citric.—Subtract 1.067 × the volatile acids, expressed as acetic, from the total acid, expressed as citric.

Determination of lactic acid.—Transfer 100 grams of the sample to a 500 cc bottle suitable for use in the centrifuge. Dilute to about 450 cc with water and add 10 cc of 20 per cent normal lead acetate. Shake well and centrifuge. Test supernatant liquid with a drop of lead acetate solution to determine whether precipitation is complete. If necessary add a few cubic centimeters more lead acetate solution, shake again, and centrifuge. When precipitation is complete dilute to 500 cc with water, mix well, centrifuge, and filter. Measure out the largest possible aliquot of the filtrate (usually 400 cc) and add a slight excess of sulphuric acid. Filter, wash the precipitated lead sulphate with a small amount of water, and evaporate the filtrate on the steam bath to a convenient volume for extraction in a continuous liquid extractor (usually 100 cc), extract for from 18 to 20 hours in a liquid extractor with washed ether (alcohol free). In case the quantity of lactic acid present is greater than 0.5 gram, it is usually necessary to extract for a longer period. In any case it is best to re-extract for from 8 to 10 hours to make sure that the extraction is complete. (Ether sufficiently pure for this purpose may be prepared by shaking out ordinary ether once with a sodium hydrate solution and then 10 times with small quantities of water.) Transfer the ether extract to a beaker, add about 20 cc of water, and evaporate the ether on the steam bath. Continue the evaporation until only about 10 cc of solution remains. Dilute to 20 cc and again evaporate to 10 cc to remove all traces of ether. Filter if necessary. To the clear solution add approximately 3 grams of sodium hydrate and exactly 100 cc of a 1½ per cent solution of potassium permanganate. Heat the mixture on a water bath at 100° C. for half an hour. At the end of that time the solution should have a decided blue-black or purple color. If the solution turns green or colorless above a layer of brown precipitate at any time during the course of the oxidation, more standard permanganate in measured portions must be added until the blue-black or purple color remains throughout a half hour of heating. The oxidation is then complete. Strongly acidify the hot solution with about 50 cc of 10 per cent sulphuric acid and run in standard permanganate from a burette until the solution is decolorized. A 5 per cent solution of oxalic acid is convenient for this purpose. Titrate back any slight excess of oxalic acid with the standard permanganate solution. Any standard solutions of potassium permanganate and oxalic acid may be used for these titrations within reasonable limits of strength. In alkaline solution the permanganate oxidizes the lactic acid quantitatively to oxalic acid according to the equation $2C_3H_6O_3+10KMnO_4=2(COOH)_2+4H_2O+2CO_2+5MnO_2+5K_2MnO_4$. Then, in acid solution, the oxalic acid is further oxidized by the permanganate to carbon dioxid and water according to the equation $5(COOH)_2+2KMnO_4+3H_2SO_4=10CO_2+8H_2O+K_2SO_4+2MnSO_4$.

Calculation.—The total weight of permanganate used in the oxidation of the lactic acid is determined by subtracting the permanganate equivalent of the oxalic acid used from the total amount of permanganate added. The weight of permanganate times 0.237 equals the weight of lactic acid.

Determination of citric acid.—Weigh 25 grams of the sample into a 250-cc beaker, dilute to approximately 200 with 95 per cent alcohol, allow to stand, with frequent stirring, for 4 hours, filter through a folded filter, and wash with 50 cc of 80 per cent alcohol. To the filtrate add sufficient water to dilute the alcohol to 50 or 60 per cent, then add 10 cc of 20 per cent barium acetate solution. Stir well with a glass rod and allow to stand overnight. In the morning filter on a Gooch crucible, wash with 50 per cent alcohol, dry for 3 to 4 hours in the oven at 100° C., and weigh. Multiply the weight of precipitate by 0.51 to obtain the weight of anhydrous citric acid.

Determination of sand.—Weigh 100 grams of well mixed sample into a 2 or 3 liter beaker, nearly fill the beaker with water, and mix the contents thoroughly. Allow to stand 5 minutes and decant the supernatant liquid into a second beaker. Refill the first with water and again mix the contents. After 5 minutes more decant the second beaker into a third, the first into the second, refill, and again mix the first. Continue this operation, decanting from the third beaker into the sink until the lighter material is washed out from the ketchup. Then collect the sand from the 3 beakers into a tared Gooch crucible, dry, ignite, and weigh. Attention is especially called to the fact that under "Sand" only the figure obtained by this method should be reported. The results obtained by the determination of ash insoluble in hydrochloric acid are not applicable to the determination of sand, and the sand is so unevenly distributed that reliable results can only be obtained by taking a larger sample than is possible in the determination of ash.

REPORT ON COCOA AND COCOA PRODUCTS.

By W. L. Dubois, *Associate Referee.*

Milk Solids in Milk Chocolate.

In the work previously reported to the association under this heading it was shown that satisfactory methods exist for the determination of lactose and butter fat in milk chocolates, the former being calculated from the polarimetric readings of a properly prepared solution of fat-free chocolate, and the latter from the Reichert-Meissl number of the extracted fat, the percentage of total fat in the chocolate under examination being known. There remained, therefore, the necessity of finding a reliable procedure for the estimation of casein.

CASEIN.

The referee reported to the association last year a test made of a French method (Ann. fals. August, 1911, p. 422), which depended on extracting the casein with sodium phosphate solution, precipitating with trichloracetic acid, and calculating the casein from the weight of this precipitate. The amount of precipitate obtained was small, and it was impossible to free it entirely from occluded cocoa material, so the results did not appear to be accurate. When applied to chocolate of known casein content, the figures prove to be approximate, however. In this year's work efforts were applied to the testing of a method proposed by Baier and Neumann (Zts. Nahr. Genussm. 1909, *18:*13), which consists in extracting the casein from the defatted chocolate by sodium oxalate and precipitating with uranium acetate in acetic-acid solution. To test this method accurately, the associate referee secured two samples of milk chocolate prepared by a well-known manufacturer, who furnished the formulæ by which the chocolates were made and also a sample of the milk powder entering into their composition. From the formulæ submitted and the analysis of the milk powder the amount of casein in the two milk chocolates was calculated. A sufficient quantity of these chocolates was defatted and sent to several collaborators for the determination of casein, the results to be reported on the material as sent. The method which accompanied these samples was as follows:

Determination of casein.—Rub 10 grams in a mortar, with the gradual addition of 1 per cent sodium oxalate solution, avoiding the formation of lumps, and pour into a 250 cc flask until 200 cc of the sodium oxalate solution have been used. Place the flask on an asbestos board and heat slowly until the contents come to a boil, occupying at least 30 minutes in bringing it to this temperature. During the heating have a funnel, the stem of which is closed by melting, rest in the neck of the flask. Fill to the mark with hot sodium oxalate solution, allow to stand, with frequent shaking, until the next day, fill to the mark with sodium oxalate solution at 20° C., mix, and filter through a folded filter. To 100 cc of the filtrate add 5 cc of a 5 per cent solution of uranium acetate and then, drop by drop, with continuous stirring, add 35 drops (or approximately 1.5 cc) of 30 per cent acetic acid. Separate the precipitate from the liquid by means of centrifuge, wash three times with a solution containing 5 grams of uranium acetate and 3 cc of 30 per cent acetic acid in 100 cc. (This washing is quickly accomplished by shaking the precipitate in the centrifuge bottle with about 75 cc of the wash solution and throwing out the solid material by centrifuge.) Finally, wash the precipitate onto a nitrogen-free filter with the washing solution and determine nitrogen in the precipitate, multiplying this result by 6.38 to obtain the casein.

Five chemists, including the referee, tested the method as submitted, and in the table below the results obtained are indicated. It will be noted that in chocolate containing the most casein the results are more accurate than in that sample containing less casein. The figures, however, are within a few tenths of a per cent of the amount actually present in both cases, and it would seem that the method promises to be sufficiently accurate to be used in the judging of the quality of the class of products to which it is to be applied.

Casein in milk chocolate.

Analyst.	Casein.				Fat in original.		Casein actually present.	
	In fat-free substance.		Calculated to original.					
	No. 1.	No. 2.	No. 1.	No. 2.	No. 1.	No. 2.	No. 1.	No. 2.
W. L. Dubois, Buffalo, N. Y.	5.50	4.84	3.68	3.24	32.61	33.55	3.91	2.82
E. Bloomberg, Galveston, Tex.	5.78	4.98	3.90	3.31	.....	.....	.....	.....
	5.79	4.92	3.90	3.27	.....	.....	.....	.....
H. E. Woodward	[1] 5.26	4.63	3.54	3.08	.....	.....	.....	.....
	5.42	4.58	3.65	3.04	.....	.....	.....	.....
L. B. Burnett, Washington, D. C.	5.59	5.32	3.77	3.54	.....	.....	.....	.....
	5.43	4.84	3.66	3.22	.....	.....	.....	.....
	5.91	5.16	3.98	3.43	.....	.....	.....	.....
W. C. Taber, Washington, D. C.	5.15	4.21	3.47	2.80	.....	.....	.....	.....
	5.28	4.74	3.56	3.15	.....	.....	.....	.....

[1] Low result probably due to solution boiling over.

COMMENTS OF COLLABORATORS.

C. S. Brinton: Mr. Hilts, who has used this method somewhat on milk-chocolate preparations, states that while the method gives good duplicates he believes that from the ratios corresponding between the lactose and casein it is doubtful if all the casein is extracted.

W. C. Taber: The first filtration of the chocolate, after being made up with the oxalate, is very slow and tedious, and it seems that this might be avoided by centrifuging as is done later on.

L. B. Burnett: The results of the samples run in triplicate do not check as well as I should expect them to.

E. Bloomberg: The determination seemed to present no difficulty, except that the filtration of the sodium oxalate solution was extremely slow.

W. L. Dubois: Oxalate solution may be centrifuged instead of filtered, thus hastening the process.

Milk Fat.

As stated above, the milk fat in chocolates is determined from the Reichert-Meissl number of the other extract. The associate referee desires to point out an error which has existed in this calculation. It is necessary to assume a constant for the Reichert-Meissl number of cocoa butter. This figure is very small. From the writer's experience, 0.5 is a close approximation to the average cocoa butter. This, in fact, is higher than most of the cocoa butters which have been analyzed by the associate referee, but is regarded as a fair figure. In making the calculation referred to, it has been the custom to subtract the constant for cocoa butter from the Reichert-Meissl number for the extracted fat, and regard the remainder as due to butter fat. In the presence of a large amount of the latter constituent this procedure is fairly accurate, but where the milk fat is present in very small amount a considerable error results from this method of calculating. The associate referee would suggest the following:

Let A=grams of butter fat.

B=grams of cocoa fat.

C=R.-M. number of extracted fat.

0.5=R.-M. number of cocoa fat.

Then,

$$\frac{24A}{5}+\frac{0.5B}{5}=C.$$

$$A+B=5.$$

$$B=5-A.$$

Working out the formula, we get this value:

$$A=\frac{C-0.5}{4.7}$$

from which the following is deduced:

$$\text{Per cent butter fat in chocolate}=\text{per cent total fat}\times\frac{C-0.5}{23.5}$$

Total Milk Solids.

Applying the above methods and the polarimetric method for lactose reported to the association at a previous meeting (Bureau of Chemistry Bul. 137, p. 98), the milk solids were determined on four chocolates, two of these being those sent to the collaborators and two others obtained from other well-known manufacturers, who stated the amount of milk solids present. In the table below appear the results showing how closely these methods determine milk constituents in milk chocolate. In these results no account is taken of the mineral constituents of the milk, which would probably raise the total solids found by approximately 1 per cent. These results are about as close as can be expected. For the Reichert-Meissl number of milk fat the factor 24 is assumed in the calculations. This, of course, gives a higher percentage of milk fat present than if a larger factor be used in the formula. There is no way of telling, when examining any particular milk chocolate, as to what the Reichert-Meissl number of the fat in the milk present would be. Twenty-four is taken in the calculation because the benefit of the doubt is given to the manufacturer, and doubtless in some cases more milk solids will be credited to him than are actually present in his product.

Milk solids in milk chocolate.

Sample.	Found by analysis.					Declared present.
	Casein.	Proteids (casein ×1.25).	Lactose.	Butter fat.	Total.	
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
B	3.39	4.00	6.55	4.79	15.58	16.25
1	3.68	4.60	6.58	6.36	17.54	17.24
2	3.24	4.05	4.89	3.23	12.17	12.41

Crude Starch.

The provisional method for this determination required that the fat be extracted from the sample prior to treating with hydrochloric acid. In some work done by the associate referee and reported to the association (Bureau of Chemistry Bul. 132, p. 136) it was shown to be unnecessary to extract the fat from the sample before hydrolysis. This point was submitted to the collaborators for testing and for an opinion. The letter and the methods submitted follow:

I am sending you, under separate cover, a sample of cocoa and a sample of plain, unsweetened chocolate for the determination of crude starch (copper-reducing matters by direct acid hydrolysis). It is desired to know whether it is necessary to extract the fat from cocoa products preliminary to the acid hydrolysis. From results obtained in the Buffalo laboratory and published in the Proceedings for 1909, page 138, it seems that that part of the provisional method for crude starch (Bul. 107, Revised, p. 255) requiring the extraction of fat is unnecessary. In order to test out this point more fully the associate referee will appreciate your carrying out the following work on both samples submitted:

(1) Transfer 4 grams to an 8-ounce centrifuge bottle of convenient shape, and shake out twice with 50 cc petroleum ether, centrifuging and drawing off the ether by suction each time. Expel residual solvent by standing bottles on steam bath for a

short time, wash the cocoa material into a 500 cc Erlenmeyer flask with 200 cc of water, and conduct hydrolysis and determination as laid down in Bulletin 107, Revised, p. 253, 8a, except that the volume is to be completed to 300 cc instead of 250 cc.

(2) Omit the extraction of fat with petroleum ether and proceed as in (1).

(3) Conduct as in (1) except that after neutralizing with sodium hydroxid add basic lead acetate solution as long as precipitation is formed, and then complete volume to 300 cc. Remove lead from an aliquot of the filtrate by dry potassium oxalate, or a mixture containing 90 per cent potassium sulphate and 10 per cent potassium carbonate, and proceed with the determination as above.

The purpose of this last determination is to ascertain whether it is necessary to clarify with lead acetate. The associate referee obtained results which are referred to in the article mentioned above, which seemed to indicate that such clarification was unnecessary, but it is desirable that these findings be confirmed by the association if possible.

Reports were received from only two collaborators. The results shown in the table, however, clearly indicate that it is unnecessary to extract the fat from cocoa products before hydrolyzing with hydrochloric acid. It is also clearly demonstrated that clarification with lead subacetate gives much lower results, and it is fair to assume that some interfering reducing substance is removed by the lead reagent. This being the case, it appears that clarification with lead is essential to accurate results. It is doubtful whether the reagent should be added until there is no more precipitate because a bulky precipitate of lead chlorid is thrown down in the presence of hydrochloric acid, which is used for the hydrolysis. How much of this precipitate is lead chlorid and how much represents interfering reducing bodies has not been determined. These latter substances may be soluble in water and capable of being removed by washing previous to hydrolyzing. This point could be settled by experiment. If it be found that washing with water removes them, clarification would then be unnecessary. The precipitate formed in this process is so bulky as to concentrate the solution so that the results are not as accurate as they would be if there were no precipitate with which to contend.

When a determination of crude starch is to be made in sweetened products, it is, of course, necessary to remove the sugar before carrying out the hydrolysis. It was thought by the associate referee that a correction might be introduced for the action of the hydrochloric acid upon the sugar present, making a preliminary determination of the latter and deducting from the amount of reducing sugars that which was due to the cane sugar present. Experiments along this line, however, show that the action of the acid upon the sugars is not uniform, so that a correction factor seems to be impracticable. It is therefore necessary to find some means of dissolving the sugar from the cocoa before submitting to hydrolysis.

The difficulty with extracting the sugar with water by washing on filter paper as described in the provisional method is that the cocoa filters so slowly that the process is very tedious, sometimes extending over such a long period that the cocoa material molds before completion. It was found impossible to shake the defatted cocoa with water and separate the latter from the insoluble material by centrifuging, owing to the extreme lightness of the fine cocoa particles, because of which they refused to deposit completely. It developed, however, that if a small amount of alumina cream were added to the mixture of defatted cocoa and water and the same thoroughly shaken and centrifuged, a clear, though somewhat colored, supernatant liquid was obtained, which could be drawn off from the residue by suction. By washing defatted cocoa containing 30 per cent of sugar three times with 100 cc of water, having added 5 cc of alumina cream with the first wash water, all of the sugar was removed. Two samples of cocoa of equal amount, one containing sugar and the other containing no sugar, when treated in this way and the residue finally hydrolyzed and copper-reducing substances determined, gave the same result, illustrating the complete removal of the sugar by this method of washing. Fifteen and sixty-four hundredths per cent was obtained on the sweetened cocoa and 15.52 per cent on the unsweetened.

Clarification with lead acetate was followed in each case, using 10 cc of the reagent. The associate referee would therefore recommend to the association for its consideration the following method for the determination of crude starch in sweetened cocoa products:

Determination of crude starch.—Transfer 8 grams of the finely divided sample to an 8-ounce nursing bottle or other receptacle which can be used in the centrifuge and shake out twice with petroleum ether to remove the fat, drawing off the solvent each time by suction. Expel the residue of the ether by allowing the bottles to remain on the steam bath a sufficient length of time. Add 5 cc of alumina cream (prepared as directed in Bulletin 107, Revised, p. 40), 100 cc of water, shake until thoroughly incorporated, and centrifuge until the supernatant liquid is clear. Draw this off and repeat the shaking with water twice. Transfer the residue in the bottle to a 500 cc Erlenmeyer flask with 200 cc of water, add 20 cc of hydrochloric acid (sp. gr. 1.125), and boil for 2½ hours. Cool, nearly neutralize with sodium hydroxid, transfer to a 300 cc flask, add 10 cc basic lead acetate, mix, complete the volume to the mark, and determine copper-reducing substances in an aliquot of the filtrate.

On one cocoa on which the referee experimented it was found that the volume of the precipitate thrown out by basic lead acetate when the method was followed as outlined above was 15 cc. This is equivalent to 5 cc on 100 cc, and the results were probably too high by 5 per cent. This point, however, has not been thoroughly worked out, and the associate referee is not in position to say that the volume of the precipitate would always be the same. In fact, it is reasonable to assume that this precipitate would vary according to the character of the cocoa under examination, so that no constant factor of correction could be applied. It was thought that it might be possible to obviate this by adding known volumes of water and of the various solutions used in the process rather than to make up to a different volume. This, however, would be cumbersome, owing to the annoyance of measuring the different solutions used, and the former method appears more desirable and is probably as accurate as is required for such a determination.

Fat.

The provisional method employs anhydrous ether for the extraction of fat from cocoa products. In the report of the associate referee to the association last year the advisability of substituting petroleum ether for sulphuric ether was pointed out, for the reason that the latter seems to extract a small amount of a substance which is not cocoa fat. Some prominent workers in this field were confronted with the same fact and succeeded in recovering from this substance a crystallized product which they decided was theobromin. Sulphuric ether has a solvent action on theobromin which petroleum ether has not. The latter, at the same time, is as perfect a solvent for cocoa butter as is sulphuric ether. Petroleum ether, furthermore, mixes with water very much less readily than does the other solvent, and consequently is less apt to extract sugar in the presence of moisture. For these several reasons the importance of substituting petroleum ether for sulphuric ether in this determination is apparent.

A short table of results was submitted to the association last year which showed that ether extracts obtained with sulphuric ether were from 0.3 to 0.6 of 1 per cent higher than those obtained with petroleum ether and that this additional material was not fat but some other substance. The associate referee did not succeed in obtaining crystals from the latter, nor identifying it further than to determine that it was not cocoa butter. It is his opinion, furthermore, that grinding and repeating the extractions is unnecessary with most cocoa materials and that a simple extraction of 5 or 6 hours would be sufficient to remove the fat as completely as necessary. With some products, such as milk chocolate, however, it might be essential to repeat the extraction, and in order to have a uniform method it is probably desirable that this

point in the procedure should be retained. In view of these facts, the following method for fat determination seems superior to that now appearing in the provisional methods:

Determination of fat.—Extract 2 grams of the finely divided material, which has been mixed with an equal volume of fine sand or asbestos, in a convenient extractor with petroleum ether, boiling at about 65° C. until no more fat is removed. This requires about 5 hours. Grind the charge and reextract for 2 hours. Allow the petroleum ether to evaporate and dry the residue at the temperature of boiling water until the weight is constant.

SUGAR.

In the report to the association for 1910 it was recommended that the alternative procedure to be substituted for the method for sugar to the point at which the direct polariscopic reading was made be further studied. Further testing of this process indicates that it is applicable to some products, but with others a very cloudy filtrate is obtained, or one which does not remain clear. There is no doubt, however, that the sugar is dissolved completely by the hot water without extracting the fat, and for those cocoa preparations where a satisfactory filtrate is obtained this optional method of making the solution can be used.

REPORT ON PRESERVATIVES.

By H. E. BARNARD, *Associate Referee.*

FORMIC ACID.

The work on food preservatives this year was confined almost wholly to a study of the detection and estimation of formic acid, which needs more study than the better-known preservatives, such as formaldehyde, benzoic acid, and the sulphites. In Germany formic acid has been used as a preservative for many years, and it has recently been shown that one firm doing an extensive business in the United States has used it in the preservation of crushed fruit and fruit sirups for the last 18 years. This fact, however, did not become generally known until the publication of the investigations made by F. L. Shannon, chemist of the Michigan Dairy and Food Department. R. F. Bacon,[1] of the Division of Foods of the Bureau of Chemistry, in 1911 referred to the increasing importance of detecting and determining formic acid in food products and gave briefly certain methods of value in its estimation.

In our laboratory McAbee, using the method given by Bacon, obtained such high results that he tried the method on samples known to be pure. Even on fresh strawberries he was able to get a reduction equivalent to 0.1 or 0.2 per cent formic acid. In Mr. McAbee's hands the method[2] used by Shannon and advocated by German chemists, which depends upon the reduction of a mercuric to a mercurous salt, was quite satisfactory, although samples of known purity gave a reduction equivalent to 0.01 or 0.02 per cent formic acid. Samples of crushed strawberries showed a reduction equivalent to 0.018 per cent formic acid, and sliced peaches gave one of 0.015 per cent. This method, although quantitative in so far as estimating reduction is concerned, is not a quantitative determination of formic acid, since other reducing agents give the same results. Shannon has, therefore, used crystallographic methods for identifying the preservative, using the following procedure:[3]

Determination of formic acid.—Steam distill about 1,000 or 1,200 cc of the sirup as in the first operation, collecting the distillate (2,500 to 3,000 cc) in a receiving flask, to which have been added about 5 cc of lead cream made by precipitating a solution of

[1] J. Ind. Eng .Chem., 1912, *4*:526; U. S. Dept. Agr., Bureau of Chemistry Cir. 74.
[2] J. prakt. chem., 1911, (2) *83*:323.
[3] J. Ind. Eng. Chem., 1912, *4*:526.

lead nitrate with potassium or sodium hydrate in the presence of phenolphthalein until a faint pink color appears and washing by decantation from eight to ten times. Shake the flask occasionally and as the lead hydrate is dissolved add a few more cubic centimeters, until all of the formic acid is combined. Concentrate the liquid in a large dish on a steam or water bath to about 50 cc. Filter and transfer to a suitable crystallizing dish and set aside in a desiccator. If formic acid was present in the original material, needlelike crystals of lead formate will form. Wash the crystals with absolute alcohol to remove any lead acetate which may be present, spread on filter paper, and dry. To the dry crystals apply the following tests:

(1) Aqueous solution will reduce silver nitrate upon warming.
(2) Aqueous solution will reduce mercuric chlorid solution upon warming.
(3) Aqueous solution will reduce platinum chlorid upon warming.
(4) To a portion of the crystals in a dry test tube add sulphuric acid and warm. Carbon monoxid is generated, which will burn in the tube with a blue flame when ignited. Further, note that the lead formate is not discolored.
(5) Transfer some of the crystals to a small distilling flask, treat with concentrated phosphoric acid, and distill. The distillate, which is formic acid, will react as follows:
(*a*) Acid to litmus and acid taste.
(*b*) Reduces silver nitrate on warming.
(*c*) Reduces mercuric chlorid on warming.
(*d*) Reduces platinum chlorid on warming.
(*e*) Is reduced to formaldehyde by magnesium and dilute sulphuric acid.

In a personal communication Brinton states that in the Philadelphia laboratory he has relied for the quantitative determination of formic acid on the reduction of mercuric chlorid. He points out that in using the qualitative tests, such as the magnesium method advocated by Bacon, the presence of formaldehyde after the magnesium reduction does not necessarily prove that formic acid has been used as a preservative. He cites an instance in which a sample of fruit product preserved with alcohol gave a positive reaction for formaldehyde. The product was a commercial one, to which it was very unlikely that formic acid had been added, and contained hydrocyanic acid in small amount. Furthermore, in the process of manufacture it may have fermented somewhat, because of which the test of formic acid might have been positive, owing to the formation of the acid as one of the products of the fermentative action of bacteria and yeasts on carbohydrates. (See Bureau of Chemistry Bul. 152, p. 119.)

FORMIC ACID IN HONEY.

The belief that the free acid of honey is principally formic acid has been general until recent investigations carried on by Merl and Röhrig (see footnotes, p. 137) and reported in various journals threw some doubt upon this fact.

Farnsteiner [1] said: The opinion has prevailed quite generally that the free acid of honey is principally formic acid. As a consequence it is asserted that formic acid exerts an essential influence upon the keeping qualities of honey—in fact, that on account of this acid honey has preservative properties. Furthermore, the justification for the claim that formic acid must be entirely harmless is drawn from the fact that honey is a useful and widely used food. This constitutes the customary argument advanced by the manufacturers of fruit sirups in which formic acid has been used and by which they endeavor to meet the arguments of those who oppose its use.

Under these circumstances I consider it of interest and importance to determine whether formic acid occurs in honey at all; and if so, in what quantities, since I concluded from an investigation conducted eight years ago that the amount of formic acid can not be present in appreciable quantity. My first task was to trace the literature on this subject from the very beginning. Thus, I found that in 1882 Vogel reported on the occurrence of formic acid in the various animal secretions and commented upon the very seldom mentioned occurrence of formic acid in honey. He found that Frommsdorff considered the acid of honey to be malic acid, Kohnoke called it lactic acid, while Martius claimed that the acid of Habana honey was formic acid. Vogel, who was the first to announce that formic acid was present in honey, based his conclusion entirely upon the fact that when distilled with water the distillate reduced silver-nitrate solution. C. Bernbeck in 1883 discussed the content

[1] Zts. Nahr. Genussm., 1908, *15:* p. 598.

of formic acid in honey and issued a warning against the assumption that formic acid is a constant component of honey, and he further expressed himself that formic acid is absolutely superfluous for preserving honey, since it could be preserved for years if kept in closed containers and in a dry, cool place.

Farnsteiner says that these few researches constitute the principal evidences upon which our views on the content of formic acid are based, and, further, that we must acknowledge that these researches are absolutely insufficient to permit of an intelligent discussion of the amount of formic acid in honey. It is quite likely that all of these authors based their conclusions on the results obtained with easily reducible silver nitrate, which is generally known to react with whole groups of organic bodies.

Farnsteiner gives a further account of his own experiments upon several different honeys by distilling in the usual manner, neutralizing the distillate, concentrating, and then treating with mercuric chlorid solution; in each case he obtained a reduction to mercurous chlorid. He emphasizes, however, the fact that the reduction of mercuric chlorid to calomel is by no means proof of the presence of formic acid, but is only proof of the presence of a volatile reducing substance in the honey. The author concludes his article by repeating that the acceptation of the presence of formic acid in honey is based on the deceptive reaction with silver nitrate. No one has as yet isolated and identified the acid of honey as formic acid. He says:

My own researches do not prove the presence of formic acid, but merely the presence of a volatile reducing substance. If formic acid is present at all, it is present in the free state in merest traces. That such traces of this acid possess appreciable preservative properties no one will concede. All attempts to pronounce formic acid harmless because of its reputed presence in honey are of no avail.

Merl [1] states:

We can not ascribe to the acid distillate of honey, which acts as a reducing agent upon certain metallic salts, the presence of formic acid, since it will doubtless lead to deception. For example, Kobert, in his Lehrbuch der Intoxicationen, 1893, page 223, says that the urine because of its content of formic acid reduces Fehling's solution. The fact is, formic acid does not reduce Fehling's solution at all.

This goes to show that the methods of identifying formic acid are indirect and faulty, and that in all probability the distribution of formic acid is not as widespread in nature as formerly believed.

Röhrig [2] reiterates that a really practical method for the detection of small quantities of formic acid is not available.

Although Farnsteiner did not positively establish the presence of formic acid in honey, the purpose of his researches was to correct the false impression that the volatile acids of honey are attributable to formic acid. He shows in raspberries the presence of formic acid, by the Wegner gasometric method. He obtained a quantity of carbon monoxid equivalent to 0.00518 gram of formic acid from 2.9 kilograms of dried raspberries. He concluded that formic acid is a natural constituent of raspberries, but that it is present in hardly recognizable quantities. He said further that the results show that it would be absurd to justify the addition of formic acid in quantities from 100 to 1,000 times as great as they are found naturally for the purpose of preserving fruits and then consider it harmless.

RECOMMENDATIONS.

In view of the doubt still existing as to whether or not formic acid is actually present in honey, fresh fruits, and vegetables, and because of the lack of information as to the quantity present, it is of importance that food chemists determine this fact. If formic acid is used as a food preservative, the amounts in which it may naturally be present must be known before it is possible to do satisfactory work on the estimation of added

[1] Zts. Nahr. Genussm., 1908, *16:* 385.
[2] Zts. Nahr. Genussm., 1910, *19:* 1.

preservatives. Your referee suggests that the methods of the qualitative and quantitative estimation of formic acid be further worked out and that during the next year the mercuric chlorid method be carefully studied to determine whether or not it is accurate and satisfactory for use as a provisional method.

Benzoate of Soda.

The work on benzoate of soda has not been continued by the collaborators on preservatives. The voluminous reports on this subject made during the last two or three years lead me to believe that the methods now available for the estimation of benzoic acid are accurate and satisfactory. In our hands, at least, the methods employed have given excellent results.

REPORT ON WATER IN FOODS.

By H. C. Lythgoe, *Associate Referee.*

Your associate referee has made, according to the recommendation of 1911, a comparison of the official method for determining water in foods with the vacuum method, using different dehydrating agents. The reagents used were sulphuric acid, phosphorus pentoxid, calcium chlorid, and zinc chlorid. The highest vacuum obtainable with the pump at my disposal was 100 mm and under higher vacua the same results would be obtained in less time. Two series of results are as follows:

Determination of water in food by two methods.

Method.	Moisture found in—			
	Coffee.	Cocoa.	Malt.	Corn meal.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Official method	3.86	3.48	6.59	11.92
Vacuum method using—				
Sulphuric acid, 8 days	4.00	3.34	6.56	.....
Phosphorus pentoxid, 8 days	3.56	3.31	6.54	.....
Calcium chlorid, 8 days	3.27	2.91	6.26	.....
Zinc chlorid, 8 days	2.41	2.00	4.81	.....
Sulphuric acid, 30 days	.....	.....	.....	12.88
Phosphorus pentoxid, 30 days	.....	.....	.....	12.01

Calcium chlorid and zinc chlorid appear to be useless for this purpose, and the prolonged use of sulphuric acid gives high results. This may be due to volatility of the acid and subsequent decomposition of the sample. Phosphorus pentoxid is apparently the most feasible of the newer dehydrating agents employed, and is not open to the objection of volatility, but the dehydrating action is slower than with sulphuric acid. H. C. McNeil, of the Contracts Laboratory, Bureau of Chemistry, proposes to use calcium carbid for this purpose.

A series of determinations was made, using sulphuric acid, phosphorus pentoxid, and calcium carbid, the results of which are as follows:

Determination of water in food using sulphuric acid, phosphorus pentoxid, and calcium carbid.

Method.	Corn meal.	Malt.	Method.	Corn meal.	Malt.
Sulphuric acid—	*Per cent.*	*Per cent.*	Calcium carbid:	*Per cent.*	*Per cent.*
3 days	9.66	6.14	3 days	8.77	5.00
6 days	10.53	6.75	6 days	9.94	5.99
14 days	11.05	7.16	14 days	11.03	6.87
Phosphorus pentoxid:					
3 days	8.64	4.96	Official method	10.51	7.07
6 days	9.83	5.94			
14 days	10.52	6.51			

From the above figures we see that sulphuric acid gives the highest figures for moisture but that calcium carbid gives nearly as high results, although the desiccation is slower. Phosphorus pentoxid gives results nearly as high as calcium carbid with about the same rapidity.

It is recommended that further study be devoted to the vacuum method of moisture determination, using different dehydrating agents.

REPORT ON HEAVY METALS IN FOODS.

By H. M. Loomis, *Associate Referee.*[1]

This being the first year that an associate referee on this subject has been appointed, there were many important lines of investigation which presented themselves, among the most important being a study of methods for the determination of tin, lead, and arsenic in foods or products entering into the preparation of foods. Food Inspection Decision 148 of the Board of Food and Drug Inspection having just appeared, prohibiting the use of copper salts in coloring foods, will necessitate a study of the methods for the determination of copper also.

Tin.

It was thought by the associate referee, and several others whom he consulted, that the most important subject to be taken up was the determination of tin. As you probably know, the various methods for the determination of tin in the presence of organic matter are tedious, and where a method of wet combustion is used it is likely to be more or less disagreeable owing to the noxious acid fumes, unless the laboratory is provided with adequate ventilation. It was thought, therefore, it would suffice to start the work on several of the methods most commonly used, with a view of recommending one or possibly more of them for provisional adoption by the association at this meeting. It was hoped that it might be possible to make a beginning on the methods for arsenic if reports came in early enough. In response to a preliminary request for cooperation, 11 analysts expressed their desire to cooperate. Accordingly the following circular letter and a sample of tin salt was sent out March 2:

INSTRUCTIONS FOR COOPERATIVE WORK.

I am sending you to-day, under separate cover, a vial of tin salt to be used in the study of methods for the determination of tin in food products for the Association of Official Agricultural Chemists. The analysis of this tin salt shows it to contain 51.98 per cent of metallic tin, and, for the sake of uniformity, I recommend that such an amount of the salt be used in determinations that the product shall contain about 200 mg per kilo of metallic tin.

[1] Presented by A. S. Mitchell.

I should advise those who have not had recent experience in the wet combustion method used in this work that it would be a good plan to make a few blank trials on the first part of the method, as, at first, there is often much difficulty in avoiding loss by frothing.

After some preliminary work and correspondence on this subject I have decided that, of the wet combustion methods, that proposed by Doolittle and Lourie is the most promising, and it seems best to compare this with the dry combustion method of Schreiber and Taber, described in Circular 67, Bureau of Chemistry, and with a modification of Allen's method, on page 61 of Bulletin 107, Revised, Bureau of Chemistry.

I trust that some of the collaborators will have the necessary facilities for the electrodeposition of the tin, so that that modification may be tried on any of the methods as well as the ignition and weighing as stannic oxid.

In connection with the Doolittle and Lourie method I beg to call your attention to the statement of Smith and Bartlett on page 137 of Bulletin 137 of the Bureau of Chemistry, where they state that some stannic salt adheres to the digestion flask, after dilution of the acid, and can not be removed by washing with water. If that is the case it may be best to rinse out the flask with some of the strong ammonia, used afterwards to neutralize the acid solution, and I think this point requires a little study on the part of collaborators.

For the study of the methods proposed I used as food materials apple sauce prepared in the laboratory and dried fish as representing fairly well products high in carbohydrates and protein, respectively. To avoid excessive frothing after the addition of nitric acid in the digestion method with the apple sauce, I found it necessary to dilute with about 200 cc of water at that point, when the mixture could then be boiled down and the digestion conducted with ease.

It seems advisable, if possible, to use at least 50 grams of material in order to make the factor in calculating to milligrams per kilo as low as possible.

METHODS.

Modification of Allen's method.—Treat 50 to 100 grams of material, dried on the water bath if necessary, in a 4-inch porcelain or fused quartz dish, with only enough concentrated sulphuric acid to char the material thoroughly. Add 5 cc of nitric acid, stir, and allow to stand until foaming has ceased. Then add 3 grams of magnesium oxid and mix thoroughly. Ignite over a Bunsen burner, or preferably in a muffle, until thoroughly charred. If necessary, grind the charred material to a powder and again ignite to complete combustion, adding a little ammonium nitrate if necessary to complete the destruction of the carbon. Transfer the ash and rinse the dish carefully into a 250-cc beaker with about 50 cc hydrochloric acid (1 to 3) and heat to boiling or on steam bath for one-half hour. Nearly neutralize the acid with sodium hydrate, dilute to 150 cc, and precipitate with hydrogen sulphid. Keep the solution in a warm place for one-half hour and filter. Dry the precipitate and fuse it with sodium hydroxid in a silver or nickel dish. Dissolve the fused mass in water, filter if necessary, make slightly acid with hydrochloric acid, and precipitate tin in the filtrate with hydrogen sulphid. The tin sulphid may be filtered, washed with hydrogen sulphid water, dried, ignited, and weighed as stannic oxid, or may be determined volumetrically as given on page 62, Bulletin 107, Revised, Bureau of Chemistry.

Method proposed by Doolittle and Lourie.—Place 25 to 50 grams of the well-mixed and finely ground sample in a Kjeldahl flask (800 to 1,000 cc) and add 25 to 50 cc of concentrated sulphuric acid, the amount depending upon the weight of the charge and the nature of the material. Place the flask on a hot plate or on wire gauze over free flame; add about 30 cc of concentrated nitric acid, raise temperature to boil, and heat till white fumes are generated, then without cooling add 10 cc of nitric acid and continue heating as before. Repeat the nitric acid addition until the solution remains clear (usually straw color) after boiling off the nitric-acid fumes. The digestion can easily be accomplished in three hours with three to four additions of nitric acid. Let the solution cool and dilute to about 400 cc with water. Nearly neutralize with concentrated ammonium hydroxid, transfer the solution to a beaker, saturate with hydrogen-sulphid gas, and let the precipitate settle on a steam bath. Filter, wash the precipitate with a little hot water saturated with hydrogen sulphid, and then dissolve it in hot yellow ammonium sulphid; reprecipitate with acetic acid or hydrochloric acid, filter on ashless paper, heat gently and carefully until carbon is burned off, moisten with nitric acid, dry, ignite, and weigh as stannic oxid (SnO_2).

NOTE.—50 cc of concentrated ammonium hydroxid will nearly neutralize 25 cc of concentrated sulphuric acid. Make usual tests for complete precipitation in the

filtrate from the first tin sulphid precipitate. In the case of canned vegetables as high as 100 grams may be taken without using more than 50 cc of sulphuric acid. With fish it is best to take as many cubic centimeters of sulphuric acid as grams of fish. The rapidity of the digestion depends on the temperature maintained—the higher the temperature, the faster the material is oxidized.

This work was planned in accordance with the recommendation of Mr. Hoagland, the referee on meat and fish for last year.

Later a letter was sent out to the cooperators asking that the use of potassium hydroxid for the solution of the tin sulphid precipitate be tried as a substitute for the disagreeable yellow ammonium sulphid, as suggested by Schreiber and Taber, beginning with "Return the filter paper and precipitate," in the second paragraph on page 7 of Circular 67, Bureau of Chemistry. This method is referred to as the "Combination method" in the table following.

Other methods suggested to the associate referee were the volumetric method used by the American Can Co., and Schryver and Buchanan's method. The former was brought to the referee's attention too late to incorporate in the cooperative work, but some encouraging experiments were made on the method by Mr. Clough, of the Seattle laboratory. Schryver and Buchanan's method was tried but abandoned, as the oxidation with sulphuric acid and potassium sulphate is much slower than with sulphuric-nitric acid mixture, and unless a large volume of sulphuric acid is used, which interferes in the later steps of the determination, the flask is very apt to be cracked by caking of the contents.

Volumetric method of the American Can Co.—Precipitate tin after wet Combustion in the usual way by hydrogen sulphid. Filter this precipitate directly on a Gooch crucible with a false bottom and wash; this is very quickly accomplished under suction. Then transfer the precipitate with the asbestos pad simply by pushing in the false bottom of an Erlenmeyer flask; digest with the addition of hydrochloric acid and potassium chlorate, dispel the chlorin by the addition of some aluminum foil. Then attach the flasks in duplicate to a Kipp apparatus, producing carbon dioxid. Raise the delivery tubes from the water seal out of the water and allow the gas to run through the Erlenmeyer flask for a few minutes. Then drop the delivery tubes to the bottom of the water seal cylinder and put two pieces of aluminum foil, weighing probably a gram or a gram and a half, into the Erlenmeyer flasks. The whole is put into solution, of course not including the asbestos. Cool the flasks in ice water and disconnect them, one at a time; wash out bulbs with the air-free water and dilute so that the hydrochloric acid will not be over about one-third strength. Then titrate as quickly as possible with one-hundredth-normal iodin, using starch paste as an indicator.

If it is desired to titrate by the excess method, an excess of one-hundredth-normal iodin diluted with the proper amount of water to take care of the acidity in the flask is added directly, while the flasks are under carbon dioxid insulation, by lifting up the cork and pouring in the iodin solution while the carbon dioxid is pouring out and preventing any contact of the air with the solution. The excess of iodin is then titrated back with one-hundredth-normal thiosulphate.

After sending out the letter of instructions, Mr. Winton called my attention to the method devised by Hansen and Johnson of the Chicago laboratory, which was read before the last meeting of the association. This method is substantially like that of Doolittle and Lourie, but for the beginning of the wet combustion dilution of the acids is required.

An important contribution to this subject is a thesis presented by E. B. Wettengel to the Massachusetts Institute of Technology, entitled "An investigation of methods recently proposed for the determination of tin in canned food products." In this thesis Wettengel discussed the following methods for the determination of tin: Those of Hehner, Wirthle, Schryver and Buchanan, Doolittle and Lourie, the American Can Co., and the last as modified by the Institute of Industrial Research. He considered the volumetric method of the American Can Co. as the most promising, and

after much work with this method and a method of wet combustion and electrolytic precipitation from ammonium sulphid solution, he came to the following conclusions:

(1) That the gravimetric methods, as employed, are not to be recommended for the determination of tin in canned foods. There are too many operations where loss of tin may occur. In the filtration of the tin sulphid loss is likely to occur from the formation of a colloidal solution, and as this filtration must be done twice this damage is doubled.

(2) That the volumetric method of the American Can Co. is reliable for the determination of tin in canned foods. This method has the advantage that the sulphid is filtered on asbestos, which is an improvement over filtering paper. The filtration of sulphid precipitate is carried out only once, which is an advantage. When the reduction is carried on so that there is no danger of oxidation, the titration by iodin will give satisfactory results. From the point of time, this method offers a decided advantage, as the whole process, in duplicate, can be finished in an hour after the destruction of the organic matter. The iodin solution can be standardized against metallic tin, if desired, instead of against arsenic trioxid.

(3) That an electrolytic method can be employed where a rotating cathode apparatus is available. This method, although longer in actual time taken for analysis, has the advantage that all electrolytic methods have, of not needing attention during the determination. The filtration of the iron and lead sulphids is rapid, and washing is accomplished quickly. The determination can be carried out in large volumes of solution as well as in small volumes. The results obtained are as accurate, if not more so, than those obtained by the other method. Where the apparatus is available, I prefer this method to that of the volumetric determination for ease of manipulation and saving of chemicals.

(4) That the large amounts of acid recommended for the digestion of the food product are unnecessary. In the case of food materials high in water content, as tomatoes, beets, spinach, and pumpkin, very small amounts of sulphuric acid, usually 20 cc or less, are sufficient, and the nitric acid may be added with the sulphuric acid at the beginning. With highly saccharine products, more sulphuric acid is necessary, but usually 100 cc will suffice. It is better to add this acid in portions rather than all in the beginning, as in case frothing does take place there is less danger of the material being ejected from the flask. In the case of fish and meats, it is best to add as many cubic centimeters of acid as grams of sample are taken. An asbestos or lead pipe connected to a suction fan into which the necks of the Kjeldahl could project would be an advantage, as then, in the case of large volumes of acid remaining after digestion, a considerable portion could be fumed away, leaving approximately 10 to 20 cc of solution.

Electrolytic method proposed by E. B. Wettengel.—To 20 cc of sulphuric acid (corresponding to the solution left after the digestion of food product), add the tin solution and dilute with 25 cc of water, add a few drops of ferric chlorid and lead acetate, and make alkaline with ammonium hydroxid. Usually 50 cc of (0.90) ammonium hydroxid are sufficient. Then add 10 cc of ammonium sulphid and 5 to 10 cc of ammonium polysulphid, to dissolve any stannous sulphid that might be present, and filter the solution on a fluted filter paper; wash, heat the filtrate to boiling, and electrolyze, using a rotating cathode (a platinum crucible) and current of 1.6 to 2.8 amperes, with a voltage of 5 to 5.5 volts.

RESULTS OF COOPERATIVE WORK.

The referee regrets to say that owing to a breakdown in the ventilating system and the many demands upon his time he was unable to do more work on the determination of tin, and he was much disappointed to receive a report from only one of the eleven who had offered to collaborate in time to insert it in this report. This collaborator was W. Alexander, of the New York laboratory, Bureau of Chemistry. R. W. Clough, of the Seattle laboratory, has also assisted with a great deal of analytical work.

Cooperative work on determination of tin in foods.

[Added amounts of tin from 200 to 700 mg per kilo.]

Analyst.	Substance.	Tin recovered.				
		Schreiber and Taber method.	Allen method.	Doolittle and Lourie method.	Volumetric method of American Can Co.	"Combination method."
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
H. M. Loomis, Seattle, Wash	Fish		73.0	47.6		
			68.2	63.5		
			66.5	80.9		
				87.9		
				68.4		
				87.3		
R. W. Clough, Seattle, Wash	do	72.7		52.8	96.2	84.9
		71.2		63.4	78.5	87.6
		91.7		78.8	97.0	
				94.0	96.6	
					95.6	
					89.2	
W. Alexander, New York, N. Y.	do		93.7	82.1		93.7
			93.0			90.6
H. M. Loomis, Seattle, Wash	Apple sauce		66.5	94.0		
			80.8			
W. Alexander, New York, N. Y.	Jam		94.5	90.6		77.2
				101.6		69.3
R. W. Clough, Seattle, Wash	Sweetened gelatin				90.5	
					88.1	
					86.8	
					90.2	
					95.6	
W. Alexander, New York, N. Y.	Tomatoes	85.1				100.7

W. Alexander made the following report on the analysis of tin salt (51.98 per cent tin) sent out by the associate referee:

(a) *With nitric acid* (200-gram sample).—(1) 51.85 per cent; (2) 51.93 per cent; average, 51.89 per cent.

(b) *Tin sulphid method.*—(a) Using an aliquot of a hydrochloric-acid solution of the salt, containing 0.020 gram of the salt; precipitated with hydrogen sulphid; dissolved with potassium hydroxid (20 per cent): Tin found—(1) 47.67 per cent; (2) 47.67 per cent; average, 47.67 per cent; recovery, 91.7 per cent.

(b) With ammonium sulphid instead of potassium hydroxid: Tin found—(1) 52.40 per cent; (2) 52.40 per cent; average, 52.40 per cent; recovery, 100.7 per cent.

COMMENTS BY ANALYSTS.

W. Alexander, New York, N. Y.: (1) Several volumetric methods were tried, but no satisfactory results were obtained. I doubt if any time can be saved, because, in any event, the sulphid precipitate has to be precipitated twice to purify it, and it is as easy to ignite as to titrate it, if not more so.

(2) I can not understand the point in connection with the sticking of tin or of tin salts to the flasks in the wet combustion method. I have digested about 80 samples of different kinds of material during the summer and have failed to see anything stick to flasks.

(3) The method outlined for digestion in the wet combustion method is wrong. I weigh a charge of 25 to 100 grams into a Kjeldahl flask (1000 cc) and add 100 to 150 cc of concentrated nitric acid and boil on the hot plate till foaming ceases and the acid boils quietly; then add 25 to 50 cc of concentrated sulphuric acid rapidly and allow the charge to clear a little; then add concentrated nitric acid a little at a time until the solution becomes light yellow after the nitric acid is expelled.

(4) Instead of filtering the hydrogen sulphid precipitate in the potassium hydroxid method on paper, I found it much better to filter on a gooch, using an asbestos mat. After heating the mat and precipitate in the 100 cc of 20 per cent potassium hydroxid in the large Erlenmeyer flask, I refiltered through a gooch as before and washed well with hot water until free from alkali. I found it next to impossible to wash the strong lye out of the paper pulp from the filter paper as directed in Circular 67.

(5) I think the ashing methods are accurate but are entirely too long. If time is to be considered at all, they may as well be disregarded, as the wet combustion method is fully as accurate.

(6) I fully agree that the potassium hydroxid method is to be preferred to the ammonium sulphid method, because it is as accurate and is neater and cleaner.

R. W. Clough, Seattle, Wash.: The volumetric method gives better results than the gravimetric methods that I have tried and is somewhat quicker, owing largely to the acceleration in filtering and washing.

Clough gives the following figures for the amounts of sulphuric and nitric acids needed and the time of digestion of 50-gram samples:

Substance.	Sulphuric acid.	Nitric acid.	Time of digestion.
	cc.	*cc.*	*Hours.*
Gelatin	20	80 to 90	2½
Fresh fish	50	110 to 130	4½ to 5½

By cold digestion overnight with nitric and sulphuric acid, about one hour was saved in hot digestion. Amount of sulphuric acid can be reduced advantageously by watching the flasks carefully at first and adding more nitric acid whenever the material begins to get dry.

In the volumetric method, in order to insure the precipitation of all the tin as sulphid, it was found best to neutralize partly with ammonium hydroxid, pass in hydrogen sulphid for a few moments, and then add ammonium hydroxid till the black precipitate of iron sulphid first formed just fails to dissolve. Then add dilute sulphuric acid to dissolve this precipitate and add 2 to 3 cc in excess. Hydrogen sulphid is then passed in as usual.

W. C. Taber, Washington, D. C.—With my experience with the wet combustion method of Doolittle and Lourie, I have found, as Smith and Bartlett indicated, that there is danger of tin remaining in the digestion flask, especially after a long digestion period. The presence of a white residue remaining in the Kjeldahl flask after washing may indicate some tin salt, but this may be removed by ammonia or alkali hydroxid solution, if preferred.

Arsenic.

It was hoped that a beginning might be made on the collaborative study of methods for arsenic and lead determinations, but owing to the unexpectedly early date of this meeting even the reports on the tin work were very meager.

For arsenic determination both the Gutzeit and the Marsh tests, in their various modifications, have their strong adherents, and while the Gutzeit test appears to be simpler in its application, the Marsh test has the advantage that the mirror yields the arsenic in a form that can be readily identified, if not actually weighed. Clark and Woodman (see Circular 99, Bureau of Chemistry) have just published the description of a modified form of the Marsh apparatus applicable for minute quantities of arsenic, giving much valuable information as to details and precautions.

Some work was done in the Seattle laboratory by R. W. Clough in the preparation of arsenic-free hydrochloric acid, which is occasionally necessary in the presence of substances forming insoluble sulphates with sulphuric acid, and the following methods were tried:

Heating hydrochloric acid with copper gauze and distilling. (Allen, Commercial Organic Analysis, 4th ed., 1909, *1:* 146.)

Bromin-sulphurous acid method. (Thresh and Porter, Preservatives in Food and Food Examination, 1906, p. 358.)

Volatilization of arsenic in stream of hydrochloric acid gas. (Prescott and Johnson, Qualitative Chemical Analysis, 5th ed., 1901, p. 60.)

Reduction with stannous chlorid and distilling. (Fresenius, Manual of Qualitative Chemical Analysis, p. 63.)

No method gave hydrochloric acid absolutely arsenic-free, but the method of Allen gave the best results, reducing the arsenic content from 1.2 mg per liter to a mere trace in all but the first 250 cc distilled.

Lead.

As for the determination of lead, interest has lately centered chiefly in its determination in cream of tartar, tartaric acid, and baking powders containing these two ingredients. The lead contamination is due to the use of lead pipes or other apparatus in the manufacture of cream of tartar and tartaric acid.

The only method used for lead determination in such products, as far as is known to the referee, is the colorimetric one given in Allen's Commercial Organic Analysis, fourth edition, 1909, volume 1, page 569.

A. F. Seeker has devised a method for the determination of lead in baking powders containing phosphate. No collaborative work has yet been done on either of these methods.

Recommendations.

(1) That a further study be made of the various modifications in the nitric-sulphuric method for destroying organic matter in the determination of tin in foods.

(2) That the use of potassium hydroxid, in place of yellow ammonium sulphid, in the solution of tin sulphids be further investigated.

(3) That a study be made of the electro deposition of tin, especially from ammonium sulphid solutions.

(4) That the volumetric method of the American Can Co., applicable for the determination of tin in foods, be made the subject of cooperative work.

(5) That the use of a Gooch crucible in filtering the first tin sulphid precipitate in the gravimetric methods be tried.

A paper on "The determination of lead in cream of tartar and baking powder" was read by Paul D. Potter, but more work is to be done on the method before the results will be published.

REPORT ON THE SEPARATION OF NITROGENOUS BODIES (MEAT PROTEINS).

By A. D. Emmett, *Referee.*[1]

In accordance with the recommendations of the association that the referee work upon meats and beef extract, a sample of desiccated lean beef round and one of high-grade beef extract were sent out to those who expressed a willingness to cooperate. The meat was desiccated according to the method of Shackell,[2] then ground and passed through a sieve. Through the kindness of Mr. Rudnick, of Armour & Co., it was possible to obtain a sample of fresh, thoroughly mixed beef extract the day before the samples were to be sent out. The extract was again mixed before shipping portions of it to the various analysts.

A preliminary outline of the method of procedure was sent to each chemist, requesting that he offer any suggestions or criticisms, and asking if it would be convenient to carry on the work at a specified time.

Instructions for Cooperative Work, 1912.

I. Meat.

Recommendation.—That in Bulletin 107, Revised, page 108, 7 (a), there be added the following sentence: "If desired, 5 to 7 grams of potassium sulphate may be added in addition to the mercury of the Kjeldahl method."

Total nitrogen.—Take from the sample 0.4 to 0.5 grams in triplicate and proceed as follows:

A. Use the official Kjeldahl method (Bul. 107, Rev., p. 108, 7 (a)).

[1] Presented by P. F. Trowbridge.

[2] Amer. J. Phys., 1909, *24:* 3.

B. In addition to using mercury in A, add 5 to 7 grams of potassium sulphate. This should be added after the frothing has ceased and the substance has started to char. Make exactly parallel determinations in both cases. Record the time required to get the solutions clear and in B the subsequent length of time that they are digested. In the case of B run a series of determinations in triplicate, with the time for digestion (after clearing up) 1½, 2½, and 4 hours.

II. BEEF EXTRACT.

RECOMMENDATION.—That the same modification (see I) of the Kjeldahl method be recognized as provisional for meat extracts. Bulletin 107, Revised, page 114, 7 (a).

Total nitrogen.—Weigh off (by difference) 3 portions of about 7 grams each into small beakers. Dissolve in cold ammonia-free water and make up to exactly 250 cc. Measure off portions of 25 cc each and proceed as outlined for meats under I. If limited for time, carry on the digestion for four hours only.

III. COLD WATER SOLUBLE NITROGEN.

RECOMMENDATION.—That the referee for next year make a further study of the separation of nitrogenous bodies (meat proteins).

Preparation of water extract.—Weigh off (by difference) 3 lots of the desiccated meat of about 7 grams each into 150 cc beakers. Add 5 to 10 cc of cold (15° C.) ammonia-free distilled water to each. Stir and make a homogeneous paste. Then add to each beaker 50 cc of the distilled water. Stir frequently for 15 minutes and then let the mixture stand for two to three minutes. Decant the liquid upon quantitative filters, having one for each beaker. Collect the filtrates in 500 cc measuring flasks. Drain the beakers, pressing out the liquid from the meat residue with the aid of the glass rod. Add to the residues in the beaker 50 cc of the cold water, stir for 5 minutes, and after letting stand two or three minutes decant as before. In case a large portion of the meat is carried over into the filters transfer it back with the aid of the glass rod. Repeat the extraction as above, using the following additional amounts of water: 50, 50, 25, 25, 25, and 25 cc. After the last extraction transfer the entire insoluble portions to the filters and wash three times with about 10 cc of water. After each extraction allow each filter to drain thoroughly before pouring on the next extract. Dilute each of the three extracts up to the mark and after thoroughly mixing them make the following determinations:

(A) *Total soluble nitrogen.*—Transfer 25 cc in duplicate from each of the three extracts to Kjeldahl flasks and proceed exactly as outlined in I, B, digesting the solution for 1½ hours after it clears up.

(B) *Coagulable nitrogen.*—Transfer 150 cc in duplicate from each of the three extracts to 250 cc beakers. Evaporate on the steam bath to about 40 cc. (If at this point the contents are not already neutral or faintly alkaline to litmus (paper) add cautiously either twentieth-normal acetic acid or twentieth-normal sodium hydrate and heat.) The coagulum should separate out, leaving a clear liquid. Filter on quantitative paper, using, if possible, 589 S. and S. "Blue Ribbon." Wash the beakers thoroughly with hot water four times, taking special care to clean the sides. Finally wash the coagulum on the filter three times and transfer it with paper to a nitrogen flask and then remove any of the material adhering to the beakers with the concentrated sulphuric acid. Use 20 to 25 cc of acid in 5 cc portions for the purpose. Heat the acid in the beakers and with the glass rod, see that every particle of the coagulum comes in contact with it, transfer to the Kjeldahl flask, and cautiously use hot water to assist the complete transfer of the coagulated protein. Add the mercury and heat on the nitrogen digester gently until the water is driven off and frothing ceases, then add 5 to 7 grams of potassium sulphate and proceed as in III A.

(C) *Creatin.*—Evaporate filtrates and washings from B to almost 5 to 10 cc. Then transfer them with the least possible amount of hot water to 50 cc measuring flasks. Keep the volume below 30 cc; add 10 cc of double-normal hydrochloric acid, and mix. Hydrolyze in an autoclave at 117° to 120° C. for 20 minutes. Remove, cool under running water, and cautiously almost neutralize with sodium hydroxid to litmus. Dilute to the mark, mix thoroughly. Make a preliminary creatinin reading to ascertain what volume to use to get a reading of approximately 8 mm. Use 10 cc of the sodium hydrate and 15 cc of the picric acid.

Calculate to creatin or creatin nitrogen. Report results on the accompanying blanks.

DISCUSSION.

The purpose in using the desiccated meat was to get a sample of flesh that would not be subject to such rapid changes as frozen or fresh meats. To carry on cooperative work in testing methods, it is fundamentally essential that the samples under exami-

nation should be very uniform. Thus far no comparative work has been carried out on the study of nitrogenous bodies in meats, in the sense that all analysts were to work upon the same sample. Even in the case of beef extracts much difficulty has apparently arisen in not sending out representative portions of the sample.

The triplicate results called for were not to be determinations made on an aliquot of a definite solution of the sample (beef extracts and water extract of the meat), but each was to be from a distinctly different portion of the original sample. In the case of the water extract of the meat duplicate determinations were called for from each of these triplicates.

Results of Cooperative Work, 1912.

MEAT.

Table 1.—*Comparative results on total nitrogen in meats.*

Analyst and determination.	Total nitrogen.							
	Kjeldahl method (digested 4 hours).	Kjeldahl-Gunning method, i. e., Kjeldahl method plus potassium sulphate (digested after clearing up).			Kjeldahl Gunning Arnold mothod, i. e., Kjeldahl method without potassium permanganate plus potassium sulphate (digested after clearing up).			Special method.
		1½ hours.	2½ hours.	4 hours.	1½ hours.	2½ hours.	4 hours.	
T. C. Trescot:	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	13.25	13.08	12.52	13.02	.....	.....	.....	[1] 13.18
2	13.25	12.74	12.58	12.92	.....	.....	.....	[1] 13.18
3	13.18	13.08	.....	12.80	.....	.....	.....	[1] 13.18
Average	13.23	12.97	12.55	12.91	.....	.....	.....	[1] 13.18
C. R. Moulton:								
1	13.42	13.15	Lost.	12.86	13.05	13.28	13.32	[2] 12.90
2	13.30	12.60	12.89	12.77	13.27	13.29	13.23	[2] 13.19
3	[3] 11.93	13.05	12.94	12.85	13.26	13.33	13.21	[2] 12.35
Average	13.36	12.93	12.91	12.83	13.19	13.30	13.25	[2] 12.81
Paul Rudnick and G. W. Trainor:								
1	13.45	13.11	13.14	13.14	[4] 12.97	[4] 13.06	[4] 12.92	[5] 12.92
2	13.45	13.17	13.17	13.11	[4] 12.92	[4] 12.97	[4] 12.92	[5] 12.92
3	13.43	13.17	13.11	13.14	[4] 12.95	[4] 12.95	[4] 12.97	[5] 12.86
Average	13.44	13.15	13.14	13.13	[4] 12.95	[4] 12.99	[4] 12.94	[5] 12.90
A. D. Emmett and L. H. Davis:								
1	13.17	12.25	12.68	13.05	13.35	13.20	13.45	.....
2	13.19	12.88	12.94	13.15	13.31	13.26	13.25	.....
3	13.30	12.22	12.82	13.26	13.30	13.24	13.35	.....
Average	13.22	12.45	12.81	13.15	13.32	13.23	13.35	.....
H. C. Sherman, C. B. McLaughlin, and Emil Osterberg: [6]								
Lean meat	13.31	.....	.....	.....	[7] 13.37	.....	.....	.....
Globulin	16.73	.....	.....	.....	[7] 16.91	.....	.....	.....
Gelatin	15.69	.....	.....	.....	[7] 15.83	.....	.....	.....
Peptone	12.81	.....	.....	.....	[7] 12.91	.....	.....	.....
White of egg	12.63	.....	.....	.....	[7] 12.70	.....	.....	.....
Dried curd	11.86	.....	.....	.....	[7] 11.86	.....	.....	.....
Average	13.84	.....	.....	.....	[1] 13.93	.....	.....	.....

[1] Gunning method used.
[2] Omitted from average.
[3] Kjeldahl method + K_2SO_4, digested from start to end, 4 hours.
[4] The samples for these determinations were weighed out several days after the others. Possibly the ow results are due to absorption from moisture. Compare with corresponding values determined by Kjeldahl method; see column Special method.
[5] Determinations made at the same time as those in (4), by Kjeldahl method.
[6] J. Amer. Chem. Soc., 1904, *26*:367.
[7] Digested 2 hours after clearing up, using Kjeldahl-Gunning method without permanganate.

The results of the different analysts for the desiccated meat show that those determined by the Kjeldahl method agree very well. When the Kjeldahl-Gunning method was followed as directed by the recommendations, that is, using sulphuric acid, mercury, permanganate, and potassium sulphate, the various data do not correspond so well. Excepting those of Rudnick and Trainor, the results were in the main very poor. They are noticeably lower than those found by the Kjeldahl method, and the triplicates do not agree well. Emmett and Davis obtained an average value very nearly as high as that for their Kjeldahl determination in the case where the digestion continued for four hours after clearing up. On the whole, the length of the period of digestion had little or no influence on the results.

Trescot ran determinations on samples of ammonium sulphate, leather, and wheat middlings. He found, as the following data show, that the addition of potassium sulphate to the Kjeldahl method gave distinctly lower results as compared with those obtained by the magnesium oxid, Kjeldahl, and Gunning methods.

Determinations on ammonium sulphate, leather, and wheat middlings.

Sample.	Time of digestion.	Nitrogen found by several methods.			
		Magnesium oxid.	Kjeldahl.	Kjeldahl + potassium sulphate.	Gunning.
	Hours.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Ammonium sulphate	3	21.05	21.05	20.07	.....
		21.18	21.18	20.63	.....
Leather	4	.....	.....	5.73	.....
		.....	.....	5.44	5.84
Wheat middlings	3	.....	.....	2.05	.....
		.....	.....	1.99	2.11

There seems to have been some misunderstanding as to what the recommendation was meant to convey. Through correspondence the referee recently learned that it was apparently intended that the recommendation should read the Kjeldahl method with potassium sulphate, but without the permanganate. This method of procedure, which Dyer [1] proposed as the Kjeldahl-Gunning-Arnold method, has already been very carefully compared with the Kjeldahl and the Gunning methods by Sherman, McLaughlin, and Osterberg,[2] and by Sherman and Falk.[3] They considered that the Kjeldahl-Gunning-Arnold method gave the highest results, worked very well, and required less time to complete oxidation. For several years we have used this method at the Illinois station with our work on meats, and Mr. Trowbridge has been using it at the Missouri station. Mr. Rudnick in his letter of transmittal stated that he did not intend to use permanganate with the potassium sulphate, and repeated the work, omitting it. He said: "It is contrary to all our experience that the Kjeldahl method should give higher results than the Kjeldahl-Gunning method." This statement referred to his data where permanganate was used. Moulton reported his results both with and without the use of permanganate, as did Emmett and Davis. Trescot followed the directions as recommended, thus omitting the series where no permanganate was used.

The data in the table show clearly that the Kjeldahl-Gunning-Arnold method gives equally as good results as the Kjeldahl method. The triplicates agree well, and in the case not only of Moulton but also of Emmett and Davis the corresponding data are very close. Further, the results for the one and one-half hour period of digestion, after clearing up, are as high as those for the four-hour period. From some of the

[1] J. Chem. Soc., 1895, *67:* 811. [2] J. Amer. Chem. Soc., 1904, *26:* 367. [3] Ibid., 1904, *26:* 1469.

determinations quoted from Sherman's investigation, the results by the Kjeldahl-Gunning-Arnold method are as high and in some cases higher than those obtained by the Kjeldahl method.

Trescot reported determinations made by the Gunning method. These agree very well with their results by the Kjeldahl method and with those of Moulton and Emmett and Davis by the Kjeldahl-Gunning-Arnold method.

Beef Extract.

Table 2.—*Comparative results on total nitrogen in beef extract.*

Analyst and determination.	Total nitrogen.							
	Kjeldahl method (digested 4 hours).	Kjeldahl-Gunning method, i. e., Kjeldahl method plus potassium sulphate (digested after clearing up).			Kjeldahl-Gunning-Arnold method, i. e., Kjeldahl method without potassium permanganate plus potassium sulphate (digested after clearing up).			Special method.
		1½ hours.	2½ hours.	4 hours.	1½ hours.	2½ hours.	4 hours.	
Trescot:	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	9.40	8.34	8.30	8.97	.....	.....	.....	[1] 9.40
2	9.45	8.10	8.70	8.81	.....	.....	.....	[1] 9.40
3	9.49	8.89	8.40	8.65	.....	.....	.....	[1] 9.40
Average	9.45	8.44	8.47	8.81	.....	.....	.....	[1] 9.40
Moulton:								
1	9.23	9.31	9.06	9.28	9.38	9.33	9.35	9.07
2	9.26	9.34	9.30	9.17	9.34	9.29	9.38	9.33
3	9.41	9.32	[2] 8.73	9.12	9.34	9.34	9.36	9.01
Average	9.30	9.32	9.18	9.19	9.35	9.32	9.37	9.14
Rudnick and Trainor:								
1	9.41	9.45	9.43	9.49	9.49	9.57	9.49	.....
2	9.49	9.45	9.41	9.45	9.53	9.53	9.53	.....
3	9.40	9.40	9.40	9.47	9.47	9.52	9.53	.....
Average	9.43	9.43	9.41	9.47	9.49	9.54	9.52	.....
Emmett and Davis:								
1	9.27	8.86	8.39	8.64	9.28	9.24	9.37	.....
2	9.23	8.51	8.79	8.58	9.34	9.32	9.34	.....
3	9.19	9.06	8.10	8.22	9.28	9.35	9.38	.....
Average	9.23	8.81	8.43	8.48	9.30	9.30	9.36	.....

[1] Gunning method. [2] Omitted from average.

The data for the beef extract sample show practically the same results as did those for meat, as to the use of the Kjeldahl method and the Kjeldahl-Gunning method with permanganate.

Rudnick and Trainor's results for the two methods compare very favorably and they obtained good triplicates. Moulton's results agree fairly well but he obtained rather poor triplicates for the Kjeldahl-Gunning method. The data of Trescot and of Emmett and Davis for the Kjeldahl-Gunning method do not agree at all well with those for the Kjeldahl method.

The values by the Kjeldahl method as determined by the different analysts are quite close. Rudnick's results, however, compare better with those of Trescot, while Moulton's compare with those of Emmett and Davis.

The data obtained with the Kjeldahl-Gunning-Arnold method show very good results in all cases. The triplicates agreed well and the corresponding values for the different laboratories are about the same. Rudnick obtained results a little higher than the others. The Kjeldahl-Gunning-Arnold values show a distinct tendency to be slightly higher than those obtained by the Kjeldahl method.

Trescot determined the nitrogen by the Gunning method and obtained results which were practically the same as those by the Kjeldahl and the Kjeldahl-Gunning-Arnold methods.

Nitrogenous Bodies in Meats.

Table 3.—*Comparative results on nitrogenous bodies in meats.*

Analyst and determination.	Nitrogen soluble in cold water.			Total nitrogen soluble in water.[1]	Soluble nitrogen coagulated.	Soluble nitrogen as creatin nitrogen.	Insoluble nitrogen.	
	Total soluble.	Coagulable.	Creatin.				In fresh substance.[2]	In total nitrogen in meat.
Cook:[3]	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	2.51	1.08	0.600	18.97	41.54	23.08	10.72	81.03
2	2.61	1.05	.605	19.73	40.38	23.27	10.62	80.27
3	2.68	1.02	.598	20.26	39.23	23.00	10.55	79.74
Average	2.60	1.05	.601	19.65	40.38	23.12	10.63	80.35
Moulton:[4]								
1a	2.55	1.09	[5] .588	19.09	41.60	[5] 22.44	10.81	80.91
1b	2.58	1.09	.579	19.31	41.60	22.10	10.78	80.69
2a	2.72	1.08	[5] .568	20.36	41.22	[5] 21.68	10.64	79.64
2b	2.60	1.10	.583	19.46	41.98	22.25	10.76	80.54
3a	2.62	1.04	[5] .561	19.61	39.69	[5] 21.41	10.74	80.39
3b	2.63	1.03	([6])	19.69	39.31	([6])	10.73	80.31
Average	2.62	1.07	.576	19.59	40.90	21.98	10.74	80.41
Rudnick and Trainor:[4]								
1a	2.70	1.05	.48	20.09	38.46	17.58	10.74	79.91
1b	2.75	1.06	.48	20.46	38.83	17.58	10.69	79.54
2a	2.69	1.09	.50	20.01	39.93	18.32	10.75	79.99
2b	2.69	1.08	.50	20.01	39.56	18.32	10.75	79.99
3a	2.79	1.21	.43	20.76	44.32	[7] 15.75	10.65	79.24
3b	2.74	1.21	.49	20.39	44.32	17.95	10.70	79.61
Average	2.73	1.12	.48	20.29	40.94	17.95	10.71	79.71
Emmett and Davis:[4]								
1a	2.58	1.09	.550	19.52	41.60	20.99	10.64	80.48
1b	2.65	1.10	.546	20.05	41.98	20.84	10.57	79.95
2a	2.63	1.06	.546	19.89	40.46	20.84	10.59	80.11
2b	2.61	1.09	.543	19.74	41.60	20.73	10.61	80.26
3a	2.64	1.10	.543	19.97	41.98	20.73	10.58	80.03
3b	2.64	1.11	.540	19.97	42.37	20.61	10.58	80.03
Average	2.62	1.09	.543	19.86	41.66	20.79	10.59	80.14

[1] Calculated from average of total nitrogen in meat as determined by Kjeldahl method.
[2] Calculated by difference—the total nitrogen minus the total soluble nitrogen.
[3] Not reported in double triplicates. Used Gunning method.
[4] Used Kjeldahl-Gunning-Arnold method.
[5] Went to dryness on steam bath after removal of coagulum.
[6] Lost.
[7] Omitted from average.

Total soluble nitrogen.—The data for the cold-water soluble nitrogen agree remarkably well. The fact that the differences between duplicate determinations on the same extract are no greater than those between the different extracts shows that the method of preparation of the water extract is workable in the hands of different chemists. The referee agrees with Mr. Cook, that 50 cc of the extract should be used instead of 25 cc in this determination.

The data show that on the average 2.61 per cent of the desiccated meat was soluble in cold water, or, in per cent of the total nitrogen in the meat, about 19.8 per cent.

Coagulable nitrogen.—The results for the coagulable nitrogen are quite good for a determination involving so many transfers. The averages vary for all cases from 1.05 to 1.12 per cent, and in per cent of the total soluble nitrogen about 41 per cent is coagulated by heat. The low values for the third extracts of Cook and of Moulton are possibly due to incomplete transfer of the coagulum from the beaker to the nitro-

gen flask. Moulton attributes it to the difficulty of getting the same degree of neutrality. It is the experience of the referee that unless one makes the solution distinctly alkaline or acid to litmus paper very little trouble will be encountered. If the solution is faintly acid or neutral the results are practically the same.[1] We have used this method for several years on our chemical studies with flesh.

The high values for Rudnick's third extract may be due to incomplete washing. In the washing of the coagulum, it will generally aid greatly, whenever the filtration becomes slow, to blow a jet of hot water on the filter so as to stir up the coagulum and separate it from the paper. There is a tendency for the coagulum to form a coating on the paper and thus prevent a good drainage.

Mr. Cook suggested that the coagulation be done in a Kjeldahl flask so as to avoid the transfer. There would seem to be two objections to this; one that it will be difficult to get the proper neutrality accurately when the solution is evaporated down, and the other that unless one has arrangement for distillation in vacuo it would take much longer to complete the evaporation on the steam bath.

At this point attention should be called to the fact that the official method [2] does not direct that the extract be evaporated to a small volume before filtering, and, further, that the neutralization be done at the beginning of the heating rather than just before filtration. It would be interesting to make a comparison of these two methods.

Creatin.—The triplicate creatin determinations run quite close for each of the laboratories, but do not compare very well among themselves. When some of the reports came in, Emmett and Davis repeated their determinations, making new water extracts, etc.; the results checked up almost exactly with those obtained the first time. As a further check, a new standard solution of potassium dichromate was made, but the differences were too slight to account for the variation. Finally another Dubosco colorimeter was tried but without any effect on the results. It would seem to the referee that in carrying out further cooperative work with creatin and creatinin, portions of the same standard solution should be sent to each analyst. There may be some question as to the purity of the potassium dichromate.

From the results obtained, the per cent of creatin nitrogen varied from 0.48 to 0.60 and formed about 21 per cent of the soluble nitrogen.

Insoluble protein nitrogen.—The official methods [3] direct to "exhaust 2 grams of the sample with cold water after extraction with ether." It would seem that it would be better to get the insoluble protein in meats by extracting the sample with water before using ether. The use of ether in the case of meats does not seem quite clear. To remove the fats, the sample should be dried first and in doing this some of the proteins are coagulated and become insoluble in water. Ether also renders some of the protein insoluble in water. The insoluble protein nitrogen could be obtained by difference, the soluble nitrogen in the sample being subtracted from the total nitrogen.

In this same connection, the determination of the coagulable proteins, the proteoses, peptones, and gelatin, and the meat bases by the official method [4] might be questioned on the basis that they are to be estimated in the cold-water extract of the ether-exhausted meat rather than in the extract of the fresh sample.

In Table 3 the data for the insoluble protein nitrogen have been calculated as suggested above. They show that 10.7 per cent of the nitrogen or 66.9 per cent of the protein in the meat was insoluble in cold water and that 80.1 per cent of the total nitrogen in the meat was in the water-insoluble form.

After receiving two of the reports, it seemed necessary to get more specific information as to the details, and consequently a set of questions was sent out to each analyst. The following comments, therefore, explain themselves.

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[1] Grindley and Emmett. J. Amer. Chem. Soc., 1905, *27* (6): 668.

[2] U. S. Dept. Agr., Bureau of Chemistry Bul. 107, Rev., p. 108 (d).

[3] Ibid., p. 108 (b).

[4] Ibid., pp. 108-109, (d), (e), and (f).

COMMENTS BY ANALYSTS.

(1) *Did you run nitrogen determinations on all reagents? If so, did you incorporate the blank in calculating the results reported?*

Trescot: All reagents used were nitrogen free.

Moulton: Yes, and incorporated in report.

Rudnick: Blanks were run and deducted from all results reported.

Emmett and Davis: Yes; results corrected for blank.

(2) *Did you use 20 or 25 cc of concentrated sulphuric acid in the digestion?*

Trescot: Always use 40 cc.

Moulton: 25 cc.

Rudnick: 25 cc.

Emmett and Davis: 25 cc.

(3) *In using the Kjeldahl method plus potassium sulphate, did you use potassium permanganate at the end? If so, do you think there was any advantage?*

Trescot: Yes.

Moulton: Tried both ways, but in regular work do not use permanganate. Obtained higher results without it.

Rudnick: Determinations made both with and without permanganate. No advantage in using it with potassium sulphate.

Emmett and Davis: Ran determinations both with and without the permanganate. Better results without it.

(4) *Do you think that there is any advantage in digesting for a longer time than one and one-half hours in the case of either the meats or beef extract? If so, what?*

Cook: It is my opinion that samples of meat and plant extracts and all samples containing amino acids and related compounds should be digested three to four hours to insure a complete cleavage of the amino radical.

Trescot: In this laboratory we never oxidize anything less than three hours.

Moulton: One and one-half hours is not long enough for beef extracts. Digest all beef extracts a minimum of four hours, using mercury, sulphuric acid, and potassium sulphate.

Rudnick: There appears to be no advantage in digesting for a longer time than one and one-half hours after digestion has become clear.

Emmett and Davis: When potassium sulphate is added to the regular Kjeldahl method digestion should continue for at least four hours, but when the Kjeldahl method without permanganate is used with potassium sulphate, one and one-half hours after clearing up is sufficient.

(5) *Did you use in distillation granulated zinc, powdered zinc, or pumice? Which do you prefer?*

Trescot: Granulated zinc.

Moulton. Granulated zinc. No preference.

Rudnick: Prefer granulated zinc.

Emmett and Davis: Prefer powdered pumice.

(6) *What indicator did you use in the titration?*

Trescot: Cochineal.

Moulton: Cochineal.

Rudnick: Congo red.

Emmett and Davis: Congo red, but checked with cochineal.

(7) *In 3, "Forms of water-soluble nitrogen in meat," how did the work proceed? Did you have any special difficulty with the water extraction, the separation of the coagulable nitrogen, or the determination of creatin?*

Cook: The work proceeded satisfactorily. No particular difficulty was encountered at any stage in the process. Creatin method worked very well; prefer autoclave to change creatin to creatinin.

Moulton: Water extraction went well. Used thymol on account of hot weather. In separation of coagulable nitrogen it was difficult to get exact and uniform reaction to litmus. Creatin results vary if sample evaporates to dryness.

Rudnick: Work proceeded very nicely indeed. No special difficulties were incurred in any of the separations or determinations.

Emmett and Davis: Encountered no difficulties. Temperature during extraction should be kept at 15° C. Coagula separated out very well, and creatin determinations were quite satisfactory.

(8) *Offer any suggestions that you feel would be an improvement upon the method of procedure in the case of the meat or beef extract and the water extract.*

Cook: 50 cc of water extract instead of 25 cc for total nitrogen might well be taken. Coagulable nitrogen determination may be made very satisfactorily in a Kjeldahl flask, thus avoiding transferring the coagulum.

Trescot: In the many hundred determinations made for nitrogen in meat and beef extract we never have any trouble in getting good results by using Gunning method and oxidizing four hours.

Moulton: We much prefer freshly precipitated magnesium carbonate for neutralization in separating the coagulable protein.

Rudnick: All weighings should be made by difference and at same time. Otherwise it would be necessary to make a moisture determination with each set of weighings.

Emmett and Davis: Double the amount of water extract taken for total nitrogen, using 50 cc. Portions of the same standard solution of dichromate should be sent out for creatin work.

Recommendations.

From the foregoing discussion it would seem that the following recommendations might well be made:

I. MEAT.

(*a*) That in Bulletin 107, Revised, page 108, 7 (a), the following sentence be not added: "If desired, 5 to 7 grams of potassium sulphate may be added in addition to the mercury of the Kjeldahl method."

(*b*) That in Bulletin 107, Revised, page 108, 7 (a), the following sentence be added to the Kjeldahl method and recognized as provisional: "If desired, 5 to 7 grams of potassium sulphate may be added in addition to the mercury of the Kjeldahl method," and that no potassium permanganate be used. This modification should be designated as the Kjeldahl-Gunning-Arnold method.

(*c*) That the length of time of digestion with the Kjeldahl-Gunning-Arnold method be studied further, with a view to ascertaining whether one and one-half hours' digestion after clearing up is as complete as when digestion is carried on four hours by the Kjeldahl or Gunning method.

II. BEEF EXTRACT.

(*a*) That the same modification of the Kjeldahl method as stated in I (*a*) be not adopted to apply to beef extracts.

(*b*) That the same modification of the Kjeldahl method as stated in I (*b*) be adopted as provisional for beef extract and called the Kjeldahl-Gunning-Arnold method.

(*c*) That further study be made as to the length of the time of digesting with the Kjeldahl-Gunning-Arnold method as stated in I (*c*), for beef extracts.

III. NITROGENOUS BODIES IN MEATS.

(*a*) That in Bulletin 107, Revised, page 108 (*b*), the following method be studied during the coming year with the idea of being made optional for determining insoluble protein:

Exhaust 7 to 25 grams of the sample (depending upon its moisture content) with 330 cc of cold (15° C.) distilled water by making 11 successive extractions, 4 of 50 cc, 4 of 25 cc, and 3 of 10 cc each. Make extract up to 500 cc and determine total soluble nitrogen in 50 cc. Deduct percentage of soluble nitrogen from the total and multiply difference by 6.25 for insoluble protein.

(*b*) That in connection with Recommendation III (*a*) a comparative study be made of the method as given in Bulletin 107, Revised, page 108, 7 (*d*), with the following method:

Evaporate 150 cc of filtrate from III (*a*) to about 40 cc, nearly neutralize to litmus (paper), having it faintly acid. Heat on steam bath 5 minutes. Filter, wash, transfer paper and contents to flask, and determine nitrogen as total nitrogen.

(*c*) That further study be devoted to the method for determining creatin and creatinin in meat and beef extract, with the idea of referring it to the association for final action in 1913.

REPORT ON THE SEPARATION OF NITROGENOUS BODIES (VEGETABLE PROTEINS).

By T. B. Osborne, *Associate Referee.*[1]

The methods at present available for quantitatively estimating proteins in vegetable tissues, or in extracts obtained from these tissues, have been subjected to so little critical study that we are not in a position to make definite recommendations regarding any of them.

Many difficulties are encountered in applying any method for a quantitative determination of vegetable proteins. Some are due to a lack of precise knowledge of the properties of the vegetable proteins and their relations to the other constituents with which they are associated and others to the physical conditions encountered which render successful extraction, filtration, and purification in many cases almost, if not quite, impossible.

In view of this state of affairs the associate referee considers that the only practical way to obtain methods effectively for determining vegetable proteins quantitatively will be to devise some plan whereby extended investigations may be conducted which are so planned as to secure the necessary information.

In the following pages is given the general nature of the problems involved and a brief outline of a plan for solving them.

Methods for estimating the proportion of protein present in vegetable tissues fall naturally into two groups:

(1) Those by which the amount of protein is determined by multiplying the nitrogen by a factor founded on the nitrogen content of the protein.

(2) Those by which the protein is isolated, as such, and weighed in the purest possible form.

Before considering any of these methods in detail, attention should be directed to the conditions under which protein substances occur in vegetable tissues and also to the properties of such proteins as have been obtained from vegetable sources.

Distribution of Proteins in Vegetable Tissues.

All the living parts of plants contain protein within their cells either dissolved in the cell sap, in a semisolid state in the protoplasm, or in a solid form, as so-called reserve protein, in particular parts of the plant, such as the seeds, roots, tubers, bulbs, and buds.

If the various types of proteins present in the tissue are to be considered, we must direct attention to the anatomical structure of the different parts of the plant, for, as in animals, the different parts of plants have different functions which involve the cooperation of different types of proteins, as well as of other substances with which these are associated. In consequence of these differences, both of structure and chemical make-up, the methods to be employed in separating the proteins from one part or another must be adapted to the conditions which prevail in each case. As a particular case in point, the proteins of the wheat kernel may be cited.

In the endosperm of this seed nearly all of the protein is present in cells which contain a large quantity of starch, very little oil and mineral matter, and only insignificant quantities of substances soluble in water. In the embryo, on the other hand, the protein is present in cells which form parts of a structure capable of enormous physiological activity and correspondingly composed of a great variety of substances intimately associated with the protein constituents. In this part of the seed are found, in comparison with the endosperm, oil instead of starch, a greater quantity of inorganic constituents, a much larger proportion of nucleic acid, and a very much greater amount of substances soluble in water. Furthermore, corresponding to the

[1] Presented by J. P. Street.

physiological activity of the embryo, in this part of the seed are enzyms which act on the various substances filling the cells and consequently causing chemical changes as soon as these are extracted with water. Since the constituents of the embryo are so combined that by their interreactions under the normal conditions of growth, the new tissues of the growing organism are formed, extracts of the embryo contain a great variety of combinations between the dissolved protein and the other constituents according to the conditions employed in making the extracts. Thus compounds of protein with nucleic acid in great variety are obtained, depending on the relative dilution, degree of acidity, and other conditions of the extract. A combination of the conditions prevailing in the embryo and the endosperm of the wheat kernel is found in the entire leguminous seed, for in this the embryo is not sharply differentiated from the endosperm.

Roots and tubers present somewhat similar conditions, for these are in a state of greater or less physiological activity, and experience shows that it is more difficult to isolate the proteins in a satisfactory condition from such tissues than from quiescent seeds from which the embryos have been removed.

Concerning the isolation of proteins from leaves, stems, and buds, or from immature plants in the growing stages, too little experience has thus far been obtained to justify any definite statements.

It is thus plain that in developing methods for an accurate and satisfactory separation of proteins from vegetable tissues extremely complicated problems arise, which have not heretofore been dealt with successfully. These facts have been here set forth somewhat at length because it appears that too little consideration has been given to these features of the problem and also because a proper consideration of them indicates the direction in which future studies must be made if we are ultimately to be in a position to make satisfactory quantitative determinations of proteins in vegetable tissues.

Properties of Vegetable Proteins.

In considering the methods used for determining the vegetable proteins, attention should be directed to some of their properties. Most of our present knowledge of these proteins has been obtained by using methods originally developed in studying proteins of animal origin. It is probable that better methods may be devised, since a large proportion of the vegetable proteins thus far studied differ from the majority of the animal proteins in many important respects. Moreover, the substances with which the plant proteins are associated are so different from those of the animal tissues that it is highly probable that other solvents than those heretofore employed can be used to advantage in extracting them from vegetable cells. Thus, for example, sodium chlorid has been almost exclusively used for extracting proteins soluble in saline solutions, but experiments which I have recently made indicate that sodium sulphite may perhaps be substituted therefor with great advantage.

In attempting to separate vegetable proteins from aqueous or saline solution by coagulation by heat, the failure of many of the known vegetable proteins to coagulate completely under conditions causing the complete separation of most animal proteins should not be overlooked. The deportment of many plant proteins toward various solvents differs greatly from that of most of the animal proteins, and often has been too little considered in applying analytical methods to their quantitative determination.

Quantitative Determinations of Vegetable Proteins.

It is important at the outset to consider carefully the value of the methods now in use and also the possibility of so far improving them as to make them of real value to those who are to use them for various purposes.

ESTIMATION OF PROTEIN BY MULTIPLYING THE NITROGEN BY A FACTOR.

For many purposes it is of great importance to know the exact amount of true protein substances present in vegetable tissues. None of the methods heretofore employed accomplish this with any approach to exactness or certainty. The general practice has been to deduct from the total nitrogen the so-called nonprotein nitrogen, determined by various methods, and multiply the remainder by a factor founded on the supposed nitrogen content of the proteins contained in the particular vegetable tissue under examination.

There are three sources of error involved in this procedure: First, it is assumed that all of the nitrogen which is not estimated as nonprotein nitrogen belongs to true proteins; second, the nitrogen content of the protein to be estimated is approximately known for only a few vegetable proteins; third, the methods of estimating the nonprotein nitrogen involve more or less uncertainty and the applicability of these methods for particular cases has not yet been established. Since neither the causes nor the extent of these errors seem to have been sufficiently considered, it will be well to discuss this subject in detail.

a. *The assumption that all of the nitrogen not estimated as nonprotein nitrogen belongs to protein substances.*—The fact that the greater part of the nitrogen extracted from plant tissues can be obtained in the form of crude protein appears to be the sole basis for this assumption. While this assumption is probably in most cases approximately correct, satisfactory evidence is still lacking as to how nearly true it is. The most direct evidence is that obtained from wheat flour.

Wheat gluten contains about 80 per cent of the total nitrogen of wheat flour, the remaining 20 per cent belonging almost wholly to proteins removed by the water used for washing out the gluten. Approximately one-half of the gluten nitrogen is soluble in diluted alcohol and has been proved to belong to the protein gliadin. After completely extracting the gluten with alcohol a residue remains which can be mostly dissolved in dilute alkali, but the turbid solution which results is so difficult to filter that great losses occur in obtaining clear solutions. Although these latter when neutralized yield a relatively large proportion of nearly pure protein, conclusive evidence that other nonprotein nitrogenous substances are also present in the gluten has not yet been secured. The most that can be said is that at least 60 per cent of the nitrogen of wheat flour is certainly protein nitrogen and that of the remaining 40 per cent most, if not all, is also protein nitrogen. That any very gross error is involved in assuming the gluten nitrogen to belong wholly to protein is improbable, but the extent of such error, if one exists, remains to be demonstrated.

If a similar assumption is applied to the nitrogen of maize, rye, or barley flours the uncertainty is even greater, for, after extracting these with saline solutions and alcohol, it has not been possible to isolate the remaining protein in any state approaching purity or in any quantity even roughly corresponding to the residual nitrogen.

All other seeds present similar difficulties so that only in the case of wheat does the evidence thus far produced really justify us in regarding our present estimations of the protein content of seeds as even approximately accurate. The matter stands even worse when we attempt to determine the true protein content of other parts of plants, for these have been the subject of so little critical study that practically no data of value are at present available.

The difficulty encountered in securing better experimental evidence is enhanced by the physical conditions presented by the cellular structure of the seed. It is extremely difficult to reduce most of these to such a degree of fineness that all of the cells are ruptured, and their contents thereby made accessible to solvents. Unless the cell walls are broken down indiffusible substances, like proteins, can not be extracted even though they may be dissolved within the cell. Furthermore, the enormous surface presented by the disintegrated tissues absorbs a relatively large proportion of

the extracted and dissolved constituents of the cells, so that it is difficult to extract these with any approach to completeness unless the quantity of solvent is very great.

If the vegetable tissue under examination contains a large proportion of nucleated cells, no inconsiderable part of the nitrogen commonly estimated as protein nitrogen may belong to nucleic acid, which has a wholly different value in nutrition than has the protein. A striking example of this is presented by the embryos of wheat, which consist almost exclusively of cells of which the nuclei form a very large part. Errors thus caused differ in extent with each particular tissue, and can only be estimated, if at all, by special investigations directed to this end.

Whether or not, besides the nucleic acid, other still unknown substances occur in plants which contribute to a greater or less extent to errors in this same direction can be learned only by future investigations. If we consider the countless number of vegetable substances of both basic and acid character which are already known and that these may easily form insoluble compounds with the amphoteric proteins, it is highly probable that in many cases more or less of the nitrogen of such substances has heretofore been counted as belonging to protein.

b. *The true factor by which the protein is estimated is only approximately known.*—From what has been already said it is obvious that no exact factor can be obtained for the mean protein content of any vegetable substance until the questions raised in the preceding section are settled. For a few of the seeds, whose proteins have been somewhat extensively studied, factors can be derived which will certainly approximate the truth more nearly than the usual $N \times 6.25$. For the great mass of vegetable substances there are, however, no data from which a better factor can be derived. Whether or not analytical results which are based on the assumption that the nitrogen belongs to protein have any real scientific value and whether this supposed protein contains an assumed percentage of nitrogen should certainly be carefully considered before further increasing the mass of data of this character already recorded.

c. *The methods of estimating the nonprotein nitrogen.*—Various methods have been proposed and some of them extensively used for estimating the nonprotein nitrogen in vegetable tissues. All of these are founded on the principle of removing the protein from the plant extracts by the addition of some precipitant applied under appropriate conditions, and then determining the nitrogen either in the precipitate or in the solution. In using such methods it is assumed that all of the nonprotein nitrogen is dissolved in the extract and that none of it is precipitated by the reagents added or absorbed by the precipitate and insoluble residue of the vegetable tissues.

It is further assumed that all of the protein is thrown out of solution by the precipitant added, which may or may not be the case. Faith in these methods is largely founded on experience with proteins of animal origin. Whether or not vegetable proteins are all similarly precipitated has not yet been established. It is quite possible, if not probable, that some of these behave very differently from the animal proteins toward the usually employed precipitants.

A further uncertainty is introduced into all such determinations by the possibility of changes induced in the proteins by the action of the enzyms with which they are associated in the vegetable tissues. There is much evidence that under certain conditions a very considerable part of the protein may be thus changed, so that, if care is not taken to avoid this, very misleading results may be obtained.

ESTIMATION OF INDIVIDUAL PROTEINS BY DIRECT WEIGHING OR BY DETERMINING NITROGEN EXTRACTED UNDER DEFINITE CONDITIONS.

It is obvious from what has already been said that it is practically impossible to isolate either the total protein, or any individual protein, from a vegetable tissue sufficiently pure and in quantity approaching that actually present to justify attempts to determine its amount by weighing. Not a little work has, however, been done with methods designed to show the proportion of the different types of proteins ex-

tracted by different solvents from some vegetable products, notably wheat flour. Most of the methods employed appear to be founded largely on investigations made several years ago by the associate referee in association with C. G. Voorhees. In applying these methods the results of later studies of vegetable proteins have not been sufficiently considered. Thus, for instance, are found determinations of gliadin in wheat flour made by shaking a definite quantity of flour with alcohol of specified strength and then estimating the amount of gliadin from the nitrogen content of the extract or from its optical rotation.

In carrying out these operations the following facts seem to have been usually overlooked: Dilute alcohol extracts, besides gliadin, proteoses, and acid compounds of albumin and globulin, the proportion depending on the volume and also on the strength of the alcohol used; the amount of gliadin extracted depends, to a great extent, on the proportion of solvent to flour; the smaller the ratio the less the gliadin extracted, owing to absorption on the surface of the undissolved part of the flour. Methods involving optical determinations of the gliadin assume that the specific rotation of this protein has been established, but an examination of the literature will show that the published data must be subjected to new study before we may be satisfied as to its correct specific rotation. Furthermore, all optical determinations of gliadin are affected to a greater or less extent by the presence in the extract of other proteins than gliadin.

Attempts to demonstrate the accuracy of such methods by showing the constancy of the results obtained under rigidly controlled conditions have, of course, no value, for they furnish no evidence that the proteins estimated are those which they are assumed to be. Uniformity of such results simply shows that they may all be incorrect to the same degree.

The associate referee is probably in part responsible for most of the attempts to determine the proportion of the different types of proteins in wheat flour, for, in the original paper by Osborne and Voorhees, a statement of such estimations was given. This estimate was never regarded by the authors as anything more than roughly approximate, the best that could be made under the circumstances, and was not stated in such a way as to lead anyone to suppose that the method then employed was suitable for the purposes of quantitative analysis.

If we recall that we have a much more precise knowledge of the properties and the approximate proportion of the proteins of wheat flour than we have of those of any other vegetable substance, and that no satisfactory method has yet been developed for quantitatively determining any one of them, it is evident that a great deal of careful study must be devoted to various factors involved in the application of such methods before satisfactory results can be expected.

The associate referee has already made a beginning of such a study of the proteins of maize and of those of hempseed, the results of which will be available in case this association deems it advisable to enter further into this subject.

These results have not been made a part of this report, as they involve too much detailed study to be of present importance. The purpose of this report is simply to present a review of the general features of the existing knowledge of the conditions affecting methods for the determination of protein in vegetable tissues.

Recommendations.

From what has just been stated it appears that we have before us an analytical problem of unusual difficulty which can be satisfactorily dealt with only by long-continued investigations carefully planned so that each point may be thoroughly studied.

Agricultural chemistry has now reached a stage where it has become necessary to know, far more accurately than we do at present, the actual protein content of the more important foodstuffs, and we should no longer be satisfied with methods which

yield results of little real scientific value. Even if it ultimately proves impossible to devise ideal methods, it is surely possible to know more definitely the limitations of the older ones. Attempts to do this should not be made with the expectation of soon securing a conventional method which can be applied in a routine manner to an unlimited number of samples, for there is at present no prospect that this can be done. The conditions presented by the various vegetable foods are so different that, in almost every case, it will be necessary to devise separate methods for each particular food.

The associate referee considers, if this subject is taken up for future action, that the investigations should be conducted in such a manner as to be a credit to this association, and recommends that some plan be adopted whereby a suitable number of well-trained men may be put in a position to devote their entire time to the study of the various questions pertaining to this subject and continue their work during several years, for much experience will be required before they can learn how to deal with these difficult problems.

An exhaustive study of the proteins of a limited number of the more important vegetable foods should be gradually extended as facilities for such work can be provided. Ultimate success can be attained only by obtaining a much more precise knowledge than we now have of the properties of the different types of proteins in each particular vegetable foodstuff; only a beginning has been made in this important field.

Such investigations as are here outlined will be expensive and tedious to conduct, and unless they are undertaken on an adequate scale and by competent men the time and money will be worse than wasted. Every fact that is definitely established respecting the protein constituents of these important products will sooner or later have its value, and I think no one will dispute that we should make every possible effort to know everything we can about them.

Investigations now in progress in my laboratory show that great differences exist in the relative nutritive value of the different seed proteins, some serving equally well for both maintenance and growth, while others serve perfectly for maintenance but fail entirely to promote growth. Thus the zein of maize, our most important vegetable protein, is, when fed alone, insufficient for either maintenance or growth, but in combination with other proteins is effective for both.

It is consequently becoming every day more and more important to know not only the actual protein content of each foodstuff, but to know the proportion of each type of protein contained therein, as well as its constituent amino acids and its relative nutritive value.

REPORT OF COMMITTEE C ON RECOMMENDATIONS OF REFEREES.

By A. L. Winton, *Chairman*.[1]

(Food adulteration and separation of nitrogenous bodies (meat proteins).)

Colors.

It is recommended—

(1) That the paper on the examination for coloring matter by the referee be given further study with a view to its adoption as a provisional method in 1913.

Approved for final action as provisional in 1913.

Saccharine Products.

It is recommended—

(1) That the method for the determination of solids in molasses and other sugar products, by means of the refractometer, using Geerlig's table of equivalents and

[1] Presented by P. F. Trowbridge.

temperature corrections, be adopted as provisional, but that the results be expressed as percentages calculated from the refractometer readings.

Approved for final action as provisional in 1913.

(2) That the suggestions of the referee for 1911 as to further study be adopted.

Approved.

FRUIT PRODUCTS.

It is recommended—

(1) That a general method of procedure based upon the uranium acetate method of Yoder be tried in the estimation of malates in cane and maple products during the coming year.

Approved.

(It is hoped that this general method will permit of a determination of malic acid in fruit products containing much color and also tartaric acid.)

VINEGAR.

It is recommended—

(1) That in method "8. Reducing sugars before inversion after evaporation," as printed in the 1911 Proceedings (Bul. 152, p. 126), the words "Again add 25 cc water and evaporate to 5 cc" be omitted, the method then reading as follows: "Evaporate 50 cc to 5 cc on the water bath. Add 25 cc of water and evaporate to 5 cc. Transfer to a volumetric flask, make up to the mark, and proceed as under method 7, using a quantity equivalent to 10 or 20 cc of sample," and that this be considered for adoption as provisional in 1913.

Approved for final action as provisional in 1913.

(2) That the method for polarization (Bul. 152, p. 126) be studied with reference to whether or not the true polarization is obtained after clarifying with lead.

Approved for further study.

(3) That Fincke's method for formic acid (see p. 81) be studied during the coming year with a view to final adoption as provisional in 1913.

Approved for final action as provisional in 1913.

(4) That Methods 6, 11, 15, 16, and 17, as printed in the 1911 Proceedings, be further studied.

Approved for further study.

11. *Ash.*—Measure 25 cc into a tared platinum dish, evaporate to dryness on the steam bath, heat in the muffle at low heat to expel inflammable gases, treat the charred portion with a few cubic centimeters of water and evaporate dry on the bath; replace in the muffle at low redness for 15 minutes and continue the alternate evaporation and heating until a white or gray ash is obtained, at no time allowing the temperature to exceed a dull red; cool in desiccator and weigh.

15. *Fixed acid.*—Measure 10 cc into a 200-cc porcelain casserole, evaporate just to dryness, add 5 to 10 cc of water and again evaporate; repeat until at least five evaporations have taken place and no odor of acetic acid can be detected. Add nearly 200 cc of recently boiled distilled water and titrate with tenth-normal alkali, using phenolphthalein. One cubic centimeter of tenth-normal alkali is equivalent to 0.0067 gram of malic acid.

16. *Volatile acid.*—Calculate the fixed acid as acetic and deduct from the total acid. Express as acetic acid.

17. *Lead precipitate.*—To 10 cc in a test tube, add 2 cc normal lead acetate (20 per cent solution), shake, and let stand one-half hour. Express as turbidity, light, medium, heavy, or very heavy.

(5) That methods 1, 2, 3, 4, 7, 9, 12, and 18, as printed in the 1911 Proceedings, be adopted as provisional.

Approved for final action as provisional in 1913.

1. *Preparation of sample.*—For microscopical examination employ the original sample, but for chemical analysis filter if turbid.

2. *Calculation of results.*—Express all results as grams per 100 cc.

3. *Specific gravity.*—Determine as directed under XIII, Wine, page 83, Bulletin 107, Revised.

4. *Alcohol.*—Measure 100 cc of the sample into a round-bottom distilling flask. Make faintly alkaline with saturated caustic soda solution, add a small scrap of paraffin, distill almost 50 cc, make up to 50 cc at temperature of sample, filter if necessary, and determine specific gravity by pyknometer or Sprengel tube. Calculate per cent by volume, or grams per 100 cc, from Table II, page 203, bearing in mind that the alcohol strength of the distillate is twice that of the original vinegar.

7. *Total reducing matters before inversion.*—Proceed according to Munson and Walker's method (Bul. 107, Rev., p. 241), using 10 or 20 cc of sample. Express results as grams of invert sugar per 100 cc. Malt vinegar should be clarified with sodium phosphotungstate.

9. *Reducing sugar after inversion.*—Proceed as under "8." After the last evaporation to 5 cc transfer to a 100 cc flask with 70 cc of water and invert by one of the methods given under "VI. General methods (c)," page 40. Nearly neutralize with caustic soda, make up to the mark, and proceed as under "7," using a quantity equivalent to 10 or 20 cc of sample.

12. *Solubility and alkalinity of soluble ash.*—Add to the above ash about 10 to 15 cc distilled water, bring to a boil, and filter through a 9 cm quantitative filter. Repeat the operation twice; transfer the ash completely to the filter paper and wash with three successive portions of hot water; dry and ignite the filter with the undissolved residue at low red heat; cool, weigh, and calculate as insoluble ash. Cool the filtrate and titrate with tenth-normal hydrochloric acid, using methyl orange as indicator. Express results as number of cubic centimeters of tenth-normal hydrochloric acid per 100 cc sample.

18. *Color—Brewer's scale.*—Read in good, reflected daylight, using 0.5-inch cell and the Lovibond scale.

(6) That the method for the determination of glycerin (Bul. 137, pp. 61–63) be adopted as provisional.

Finally adopted as provisional.

Flavoring Extracts.

It is recommended—

(1) That the method for the detection of capsicum in ginger extract (Cir. 90, p. 13), as proposed by Doyle, modifying the La Wall method, be adopted as provisional. (The Doyle method is not essentially different from La Wall's. The details of procedure, however, are such as to make the test more positive and are set forth more clearly than in the latter method.)

Finally adopted as provisional.

(2) That the limits of composition of standard vanilla extracts be referred to the Committee on Food Standards for such action as they think advisable.

Approved.

(3) That the study of the composition of pure commercial vanilla extracts be continued next year for the purpose of securing additional data.

Approved.

(4) That Wichmann's method for detecting coumarin (Cir. 95) be further studied.

Approved for further study.

(5) That the study of the method (Bul. 137, p. 79) for the examination of ginger extracts and other flavoring materials, as recommended last year, be made the subject of further work next year.

Approved.

Spices.

It is recommended—

(1) That an effort be made to devise methods other than microscopical for detecting an excess of seeds in paprika.

Approved.

(2) That, if possible, samples of prepared mustard of known composition be submitted to collaborators for determination of crude fiber by the present official methods.

Approved.

Baking Powder.

It is recommended—

(1) That the study of methods for detecting arsenic and lead in baking powder ingredients be continued.

Approved.

Meat and Fish.

It is recommended—

(1) That on page 106 of Bulletin 107, Revised, under "XVII. Methods for the analysis of meat and meat products. 1. Identification of species—Provisional," fourth line, after "melting point," "melting point of stearin by Belfield-Emery method" be inserted.

Approved for final action as provisional in 1913.

(2) That Mayerhofer's method, modified (Bul. 107, Rev., p. 109), and Price's method (see p. 97), for the detection or determination of starch be studied during the next year.

Approved for further study.

(3) That Folin's method by aeration, as modified (see p. 99), be substituted for the method for ammoniacal nitrogen in animal substances under "(e) Ammonia.—Provisional," Bulletin 107, Revised, page 115.

Approved for final action as provisional in 1913.

(4) That the foaming difficulty of the aeration method be further studied.

Approved for further study.

(5) That the following distillation method be studied: Mix 25 grams of the fine sample with 5 grams of salt and 1 gram of sodium carbonate, and 100 cc of alcohol, in a 500 cc flask, and pass through it a current of vapor from boiling alcohol. Distill a 200 cc portion and titrate and then treat similarly two 100 cc portions. The last portion should contain very little ammonia.

Approved for study.

Fats and Oils.

It is recommended—

(1) That the Emery method for the detection of added beef fat in lard (U. S. Dept. Agr., Bureau of Animal Industry Cir. 132), reported at the 1911 meeting, be made provisional.

Approved for final action as provisional in 1913.

(2) That final action be taken to make the provisional method for the preparation of samples (Bul. 107, Rev., p. 129) official.

Finally adopted as official.

(3) That method "(c) Zeiss Butyro-Refractometer" (Bul. 107, Rev., p. 132) be finally adopted as official.

Adopted, final action.

(4) That method "12. Free fatty acids.—Provisional" (Bul. 107, Rev., p. 142) be finally adopted as official.

Adopted, final action.

(5) That the Halphen reaction for cottonseed oil (Bul. 107, Rev., p. 144, 17 (b)) be finally adopted as official.

Adopted, final action.

(6) That the Baudouin test for sesame oil (Bul. 107, Rev., p. 146, 17 (e)) be finally adopted as official.

Adopted, final action.

(7) That the Villavecchia test for sesame oil (Bul. 107, Rev., p. 146, 17 (f)) be finally adopted as official.

Adopted, final action.

(8) That any cut appearing in the text of the chapter on fats and oils, Bulletin 107, Revised, be considered merely as an illustration and not as an integral part of the method.

Adopted, final action.

(9) That 75° in addition or instead of 100° C. be adopted provisionally with a view to ultimately making it official for the determination of the specific gravity of high melting point fats.

Approved for final action as provisional in 1913.

(10) That the glycerol saponification method for the preparation of fatty acids for use in the titer test, as given in the associate referee's report (see p. 117), be adopted as provisional.

Approved for final action as provisional in 1913.

(11) That action on the Bechi or silver nitrate test for cottonseed oil as an official method be deferred.

Approved.

Dairy Products.

It is recommended—

(1) That the method proposed in 1911 (Bul. 152, p. 101; Cir. 90, p. 10) as applied to milk, evaporated milk, sweetened condensed milk, thin cream, and ice cream, be given further study.

Approved for further study.

(2) That for rich cream the following modification be given further study: Add 50 grams of the material, stirring vigorously, to 25 cc boiling Soxhlet solution, in a 250 cc beaker. Cover the filter with a thin layer of fibrous asbestos mixture, carefully covering the sides as far up as possible. Wash once or twice with cold water, and proceed as in the original method.

Approved for further study.

Cereal Products.

It is recommended—

(1) That the method of Bryan, Given, and Straughn (Cir. 71) for soluble carbohydrates be given a further trial.

Approved for further study.

(2) That methods for the estimation of moisture by the use of the vacuum oven and vacuum desiccator, for estimating the acidity of the water extract of flour, and Olsen's method for dry gluten be referred to the next referee for immediate consideration.

Approved for study.

(3) That the methods for the determination of nitrous nitrogen (Bul. 152, p. 113) be finally adopted as provisional.

Finally adopted as provisional.

(4) That method B for the determination of ash (Cir. 90, p. 12) be made a provisional method.

Not considered. (Available for final action as provisional in 1913.)

(5) That the method for the determination of ether extract given in Bulletin 107, Revised, page 39, 5, (b), (1), be made official.

Not considered. (Available for final action as official in 1913.)

Condiments Other than Spices.

It is recommended—

(1) That the methods for the determination of lactic acid, citric acid, insoluble solids, and sand (see pp. 128–129) be made provisional.

Approved for final action as provisional in 1913.

(2) That the committee on editing the new edition of the methods of analysis make provision for minor changes suggested by the referee in methods for the determination of total solids, soluble solids, ash, alkalinity of ash, sodium chlorid, reducing sugars before inversion, reducing sugars after inversion, sucrose, polarization after inversion, total acids as citric, volatile acids as acetic, fixed acids as citric, and detection of butyric acid, so that they may be used as optional methods.

Approved.

(3) That the Boyle method for the determination of turmeric in prepared mustard be given trial.

Approved for study.

Boyle method.—(Preparation of samples of prepared mustard with known amounts of turmeric to serve as standards.) Rub 5-gram samples of the standards and a 5-gram sample of the mustard under examination in a mortar with 50 cc of 95 per cent alcohol and transfer to 100-cc flasks. Shake these flasks occasionally during about one hour, make up to the mark with 95 per cent alcohol, and filter. Place 1 cc of each filtrate in a 50-cc beaker, hang a strip of filter paper about 1 inch wide in the beaker so that it just touches the bottom. The alcoholic solution is then absorbed by the paper, which is allowed to dry in the air. The depth of color is then compared with the standards.

Cocoa and Cocoa Products.

It is recommended—

(1) That the method for the determination of casein in milk chocolate as studied by the association this year (Bul. 152, p. 163) be adopted as provisional.

Approved for final action as provisional in 1913.

(2) That the method of determining milk fat in milk chocolate (Bul. 152, p. 159), as studied by the association for two years and as reported this year, be adopted as provisional.

Approved for final action as provisional in 1913.

(3) That the methods for crude starch in sweet and unsweetened products be further studied by the referee next year.

Approved for further study.

(4) That petroleum ether be substituted for sulphuric ether as a solvent in the determination of fat in cocoa products. The committee recommend that this be not adopted.

Recommendation of committee adopted.

Tea and Coffee.

It is recommended—

(1) That the referee for the coming year continue the lines of work as laid down by previous referees.

Approved.

Preservatives.

It is recommended—

(1) That the methods of qualitative and quantitative estimation of formic acid be further worked out, and that the mercuric chlorid method be carefully studied to determine its accuracy.

Approved for study.

Water in Foods.

It is recommended—

(1) That further study be made of the vacuum method (Bul. 122, p. 219) of determining moisture in food, using different dehydrating agents.

Approved for further study.

Organic and Inorganic Phosphorus in Foods.

It is recommended—

(1) That the referee for the coming year continue the lines of work as laid down by previous referees.

Approved.

Heavy Metals in Foods.

It is recommended—

(1) That a further study be made of the various modifications of the nitric-sulphuric acid method for destroying organic matter in the determination of tin in foods.

Approved for further study.

(2) That the use of potassium hydroxid, in place of yellow ammonium sulphid, in the solution of tin sulphids be further investigated.

Approved for further study.

(3) That a study be made of the electro deposition of tin, especially from sulphid solutions.

Approved for study.

(4) That the volumetric method of the American Can Co. (see p. 141), applicable to the determination of tin in foods, be made the subject of cooperative work.

Approved.

(5) That the use of a Gooch crucible in filtering the first tin sulphid precipitate in the gravimeteric methods be tried.

Approved.

Distilled Liquors, Wine, and Beer.

It is recommended—

(1) That the referees for the coming year continue the lines of work as laid down by previous referees.

Approved.

Separation of Nitrogenous Bodies (Meat Proteins).

I. MEATS AND BEEF EXTRACTS.

It is recommended—

(1) That in Bulletin 107, Revised, page 108, 7 (a), the following sentence be added to the Kjeldahl method and recognized as provisional: "If desired, 5 to 7 grams of potassium sulphate may be added in addition to the mercury of the Kjeldahl method, and no potassium permanganate be used." This modification should be designated as the Kjeldahl-Gunning-Arnold method.

Approved for final action as provisional in 1913.

(2) That the length of time of digestion with the Kjeldahl-Gunning-Arnold method be studied further, with a view of ascertaining whether 1½ hours' digestion after clearing up is as complete as when digestion is carried on 4 hours by the Kjeldahl or Gunning method.

Approved for further study.

II. NITROGENOUS BODIES IN MEATS.

It is recommended—

(1) That in Bulletin 107, Revised, page 108, 7 (b), the following method be studied during the coming year with the idea of its being made optional for determining insoluble protein: Exhaust 7 to 25 grams of the sample (depending upon its moisture content) with 330 cc of cold (15° C.) distilled water by making 11 successive extractions, 4 of 50 cc, 4 of 25 cc, and 3 of 10 cc each. Make extract up to 500 cc and determine total soluble nitrogen in 50 cc. Deduct percentage of soluble nitrogen from the total and multiply difference by 6.25 for insoluble protein.

Approved for final action in 1913.

(2) That in connection with Recommendation II (1) a comparative study be made of the method as given in Bulletin 107, Revised, page 108, 7 (d), with the following method: Evaporate 150 cc of filtrate from II (1) to about 40 cc and nearly neutralize to litmus (paper), having it faintly acid. Heat on steam bath 5 minutes. Filter, wash, transfer paper and contents to flask, and determine nitrogen as "total nitrogen." In both methods use the same volume of the filtrate from II (1) and bring to same neutrality.

Approved.

(3) That further study be made of the method for determining creatin and creatinin in meat and beef extract, with the idea of referring it to the association for final action in 1913.

Approved for final action in 1913.

A motion was made by Dr. Bigelow and carried by the association that the incoming president appoint a committee consisting of six members, who shall have authority to combine the methods of Bulletin 107, Revised, and the methods that have been adopted provisionally and officially since the issue of that bulletin and submit the manuscript to the Bureau of Chemistry for publication without waiting for the work to be approved by the association. The following committee on editing methods of analysis (Bulletin 107, Revised) was appointed: J. K. Haywood, of District of Columbia, chairman; W. A. Withers, of North Carolina; J. P. Street, of Connecticut; A. F. Seeker, of New York; G. W. Hoover, of District of Columbia; and B. L. Hartwell, of Rhode Island.

Adjourned.

THIRD DAY.

WEDNESDAY—MORNING SESSION.

REPORT ON DAIRY PRODUCTS.

By Lewis I. Nurenberg, *Associate Referee*,[1] in collaboration with Hermann C. Lythgoe.

My work during the past year as associate referee on dairy products has been on tests for distinguishing between pasteurized and raw milk, and also on certain reactions for detecting old milk. About 2,500 samples of commercial milk were examined. Three methods were used for detecting pasteurized milk.

Methods for Detecting Pasteurized Milk.

Schardinger test.[2]—Mix 20 cc of milk in a test tube with 1 cc of a solution containing 5 cc of a saturated alcoholic solution of Methylene Blue and 5 cc of 40 per cent formaldehyde and 190 cc of water. Cover the contents of the tube with a layer of liquid petroleum to prevent access of air, and place the tube in the water bath at a temperature of 45° to 50° C. Raw milk will decolorize this reagent in less than 20 minutes. Pasteurized milk will take longer than 20 minutes.

Rothenfusser test.[3]—Dissolve 1 gram of pure paraphenylene diamin hydrochlorid in 15 cc of water and 2 grams of crystallized guaiacol in 135 cc of 96 per cent alcohol. Mix these solutions and keep in an amber-colored bottle. To 10 cc of milk add 0.5 cc of this reagent and 3 drops of 3 per cent hydrogen dioxid. With raw milk a blue-violet coloration is formed. If the milk has been heated to a sufficiently high temperature no color is produced.

Benzidine test.[4]—Dissolve 4 grams of benzidine in 100 cc of 96 per cent alcohol. To 10 cc of milk add 1 cc of this reagent, 3 drops of 30 per cent acetic acid and 2 cc of 3 per cent hydrogen dioxid. A blue coloration is produced with raw milk, and no coloration if heated to a sufficiently high temperature.

Method for Detecting Old Milk.

Alcohol precipitation method.—For the detection of old milk a 68 per cent alcohol containing 0.3 gram alizarin was used. To 5 cc of milk add 5 cc of the reagent. A precipitate varying in color from red to yellow is indicative of the acidity of the milk. Milk, however, will give a precipitate long before it reaches the point of curdling.

In order to determine the influence of time and temperature of pasteurization on these tests, a quantity of raw milk of known purity was procured. Five 1-pint portions were heated gradually (rise in temperature about 2° per minute) in a water bath to temperatures of 60°, 65°, 70°, 75°, and 80° C. The samples heated to 60° or more were held at those temperatures for 30 minutes, and portions removed every 10 minutes. Portions were taken out also when the temperature reached 40° and 50° C. All these portions were subjected to the Schardinger, benzidine, and Rothenfusser tests and the bacterial count was made on some of them. The results, including the examination of a sample of commercially pasteurized milk which had been heated

[1] Presented by E. M. Bailey.

[2] Zts. Nahr. Genussm., 1902, *5*: 1113.

[3] Milchwirtschaftliches Zentralblatt, 1910, *6*: 468–470.

[4] Peters and Wilkinson, Zts. Nahr. Genussm., 1908, *16*: 3, 172–174.

to a temperature of 63° C. and held there for 35 minutes, are shown in the following table:

Influence of time and temperature of pasteurization on three tests for distinguishing pasteurized and raw milk.

[I=Schardinger test (Formalin Methylene Blue); II=Benzidine reaction; III=Rothenfusser reaction.]

Temperature at which raw milk was pasteurized.	Minutes required for decolorization of solutions when held—																			
	0 minutes.				10 minutes.				20 minutes.				30 minutes.				35 minutes.			
	I.	II.	III.	Bacteria per cc.	I.	II.	III.	Bacteria per cc.	I.	II.	III.	Bacteria per cc.	I.	II.	III.	Bacteria per cc.	I.	II.	III.	Bacteria per cc.
°C.																				
20	5	+	+	18,000	.....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
40	5	+	+	180	.....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
50	6	+	+	120	.....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
60	7	+	+	.....	8	+	+	....	9	+	+	....	10	+	+	60	....	....	....	....
63	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	—	+	+	....
65	9	+	+	.....	12.5	+	+	....	16	+	+	....	21	+	+	....	....	....	....	....
70	12	[1]+	+	40	20	[2]+	[1]+	....	85	[2]+	[1]+	....	[3]—	[2]+	[1]+	30	....	....	....	....
75	40	—	—	.....	[3]—	—	—	....	[3]—	—	—	....	[3]—	—	—	....	....	....	....	....
80	[3]—	—	—	(4)	[3]—	—	—	....	[3]—	—	—	....	[3]—	—	—	(5)	....	....	....	....

+ =Color produced.
— =No color produced.
[1] Faintly.
[2] Very faintly.
[3] No decolorization in 5 hours.
[4] Spores only.
[5] Sterile.

From the foregoing table the following conclusions are drawn: Milk pasteurized at 63° C. and held there for 35 minutes, or at 65° and held at that temperature for 30 minutes, or at 70° C. and held there for more than 10 minutes, or milk pasteurized at temperatures above 70° C., can be detected by means of the Schardinger reaction. Milk pasteurized at 75° C. or above can be detected by means of the Rothenfusser and benzidine reagents, but when pasteurized below 75° C. these reactions do not detect pasteurization, and therefore fail in their practical value.

To determine the effect of the addition of varying amounts of raw milk to milk pasteurized at different temperatures, 5, 10, 15, 20, and 25 per cent of raw milk was added to milk pasteurized at 60°, 65°, 70°, 75°, and 80° C., with the following results:

Effect on decolorization when raw milk is added to pasteurized milk.

(Schardinger reaction.)

Temperature at which milk was pasteurized.	Per cent raw milk added—					
	0	5	10	15	20	25
	Minutes required for decolorization of the solution.					
°C.						
60	10	10	9½	9½	9	9
65	21	15	12	.....	8	7
70	—	—	70	35	20	15
75	—	—	—	.....	40	25
80	—	—	—	—	—	25

— =No decolorization in 5 hours.

The addition of 10 per cent of raw milk was found sufficient to give the positive color with the benzidine and Rothenfusser reagents. In the case of the Schardinger reaction with milk pasteurized at 70° C. 20 per cent of raw milk can be added, and with

milk pasteurized at 75° and 80° C. amounts up to 25 per cent raw milk can be added without vitiating the test.

For the purpose of determining whether the reaction for raw milk returned after allowing pasteurized milk to stand for a few days, the following experiment was undertaken: Three samples of freshly pasteurized milk were obtained from the pasteurizer of a milk concern. This milk had been pasteurized at 63° C. and held there for 35 minutes. The samples were kept in the refrigerator and a new bottle was opened each day. The results are given in the following table:

Results on milk pasteurized at 63° C. and held for 35 minutes.

Time.	Schardinger reaction.	Benzidine reaction.	Rothenfusser reaction.	68 per cent alcohol.
First day.....	[1] —	Faintly +	+	No precipitate.
Second day..	[1] —	+	+	Do.
Third day....	10 minutes.	+	+	Do.

[1] No reduction in 1 hour. +=color produced. —=no color produced.

Here it may be concluded that three days after pasteurization the Schardinger reaction is the same as for raw milk. The benzidine and Rothenfusser tests are inclined to be more intense than that given by raw milk. None of these methods will distinguish commercially pasteurized milk three days after pasteurization and only the Schardinger test will detect it up to and including the second day.

Out of a total of 2,470 commercial samples of milk collected by the inspectors of the Massachusetts State Board of Health, 2,132 gave the test for raw milk, and 348 were pasteurized according to the Schardinger reaction. In the case of the Rothenfusser and benzidine reactions, all but 7 of this number gave the tests for raw milk. From personal knowledge of the condition of the market and the localities from which the milk came, this number is far too small. It may be concluded from this that the Schardinger test is the most feasible, while the benzidine and Rothenfusser reactions are of no practical value for the detection of commercially pasteurized milk.

The Schardinger test, using the reagent free from formaldehyde (5 cc saturated alcoholic solution of Methylene Blue 190 cc water), has been recommended as a means of detecting old milk by the rapidity of decolorization. Most authorities agree that milk which decolorizes this reagent within an hour is too old for consumption.

The obtaining of a precipitate by the addition of an equal volume of 68 per cent alcohol to milk has been used extensively as a means of detecting old milk. W. Morres[1] recommends the addition of alizarin to the alcohol, thereby giving an indication of the acidity of the milk. Both of these reactions were studies in the examination of market milk. The concentration of the alizarin was 0.3 gram per liter of 68 per cent alcohol. The colors produced in milk corresponding to different degrees of acidity varied from lilac red with fresh milk to yellow in sour milk, with several intermediate colors. The following table of Morres illustrates the reaction.

Tests using alcohol to which alizarin had been added.

Acidity, degrees. Soxhlet-Henkel.	Color of alizarin.	Condition of precipitate.	Condition of milk.
7	Lilac red	No precipitate	Normal and fresh.
8		Very fine precipitate	Beginning to sour.
9	Brownish red	Fine precipitate	Increasing sourness.
10	Reddish brown	Precipitate	Do.
11	Brown	Heavy precipitate	Critical state.
12	Yellowish brown	Very heavy precipitate	Coagulated on heating.
14	Brownish yellow	do	Do.
16 and above	Yellow	do	Do.

[1] Zts. Nahr. Genussm., 1911, *22*:459.

About 2,600 samples of commercial milk have been examined, of which 63 gave precipitates with 68 per cent alcohol. About 300 samples, many of which have been purposely kept in the laboratory for some time, have been examined. Of these 49 gave precipitates with alcohol, and 77 decolorized the Methylene Blue solution within an hour. Tabulation of the results obtained gives the following average relations between the reduction and precipitation test.

Time of reduction of Methylene Blue.	Color of precipitate.
30 minutes and above	No precipitate.
25 minutes	Reddish brown.
20 minutes	Brownish red.
15 minutes	Brown.
10 minutes	Brownish yellow.
5 minutes and less	Yellow.

The reduction of the Methylene Blue solution is said to be due to bacterial action. This test then might also serve as an indicator in the selection of milk samples high in bacteria.

The alcohol precipitation method, while not quite so delicate as the Schardinger test, is certainly more easily performed than the determination of acidity, and would be applicable for field work.

I recommend that further work be done on the Schardinger tests (formalin Methylene Blue solution and Methylene Blue solution) and the 68 per cent alcohol precipitation method.

REPORT ON FOODS AND FEEDING STUFFS.

By W. J. Jones, Jr., *Referee*.[1]

The following subjects were assigned for investigation during the past year:

(1) Continued study of the method for determining acidity and reporting results proposed by the previous referee.

(2) Continued study of the petroleum ether method for the determination of fat.

(3) Continued study of the proper factor for converting nitrogen into protein.

It was found impossible to take up the third subject, owing to the length of time required for its investigation.

Cooperative Work of 1912.

For the first two subjects eight samples, representing seven classes of feeding stuffs having a general sale, were selected, as follows:

DESCRIPTION OF SAMPLES.

(1) Continental gluten feed, composed of the dried residue from the distillation processes in the manufacture of liquor and composed of corn, oat malt, and barley malt, containing 29.55 per cent of crude protein.

(3) Choice cottonseed meal containing 42.62 per cent of crude protein.

(5) Molasses feed, composed of alfalfa, corn, oats, brewers' grains, and molasses, containing 10.64 per cent of crude protein.

(10) Linseed meal, containing 33.37 per cent of crude protein.

(16) Gluten feed composed of corn by-products and steep water from the manufacture of starch, containing 23.25 per cent of crude protein.

(19) Gluten feed, composed of corn by-products from the manufacture of starch without the steep water, containing 24.39 per cent of crude protein.

(20) A compounded feed, composed of wheat and corn gluten feed, hominy feed, barley feed and sprouts, distillers' grains, and cottonseed meal, containing 25.01 per cent of crude protein.

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[1] Presented by W. D. Bigelow.

(25) Wheat bran with a small percentage of screenings, containing 16.03 per cent of crude protein.

The samples were carefully prepared according to the official methods under the supervision of Mr. F. D. Fuller and sent to collaborators with the following instructions:

INSTRUCTIONS.

Owing to unforeseen delays it has been found impossible to take up the work of studying the proper factor for the conversion of nitrogen into protein, and the cooperative work will be limited to the study of the methods of determining and reporting results on acidity and comparison of the official and petroleum ether methods for the extraction of fat.

Eight samples representing seven classes of feeding stuffs are being sent to you by prepaid express.

Methods.

Moisture.—Determine moisture in all the samples by the official method, Bureau of Chemistry Bulletin 107, Revised, page 38. Please state amount taken, whether determination is made in vacuo or hydrogen, and temperature of bath.

Acidity.—Weigh 10 grams of the sample into a shaking bottle, add 200 cc of distilled water, and shake for 15 minutes. Filter the extract through a folded filter and take an aliquot of 20 cc (equal to 1 gram of sample) for the titration. Dilute with 50 cc of distilled water and titrate with standard deci-normal sodium hydroxid solution, using phenolphthalein as an indicator.

Free inorganic acids.—To 20 cc of the water solution diluted with 50 cc of distilled water add Methyl Orange as an indicator. Report as neutral, acid, or alkaline. If alkaline, titrate with standard deci-normal hydrochloric acid solution.

Solution.—For the purpose of uniformity in the work on acidity it is suggested that a 250 cc shaking bottle and a continuous shaking apparatus be used for securing the water solution. In case a shaking apparatus is not available, please give details of method used in securing the solution.

Fat.—(1) Official method: Determine the crude fat by the official direct method, taking care to use absolute ether in making the extraction; see Bureau of Chemistry, Bulletin 107, Revised, (a) and (b1), p. 39.

(2) Petroleum ether method: Determine the fat by extracting 2 to 5 grams of the sample, without previous drying, for three hours in a Soxhlet apparatus with petroleum ether boiling under 65° C. Then evaporate off the ether, weigh the residue, and report as oil.

For the purpose of uniformity it is suggested that 2 grams be used in all fat extractions.

Indicators.

Phenolphthalein.—Dissolve 1 gram of phenolphthalein in 100 cc of 50 per cent alcohol.

Methyl Orange.—Dissolve 0.1 gram of Methyl Orange in 100 cc of distilled water.

Reports.

Acidity.—In reporting the acidity state the results in terms of cubic centimeters of sodium hydroxid used or its equivalent in grams of sodium hydroxid.

Free inorganic acids.—Report as acid, neutral, or alkaline. If alkaline, report in terms of cubic centimeters of hydrochloric acid used.

Fat.—If amounts other than 2 grams are extracted, report amounts used with determinations of fat.

Report individual determinations, not averages.

Comparative determinations on other samples, together with suggestions and comments on work and methods, will be greatly appreciated. Kindly report results to referee as soon as possible.

COMPARATIVE RESULTS ON METHODS FOR DETERMINING ACIDITY AND ALKALINITY.

TABLE 1.—*Cooperative results on acidity of water extract with phenolphthalein as indicator.*

[Basis, 1 gram.]

Analyst.	Tenth-normal sodium hydroxid.							
	Sample No. 1.	Sample No. 3.	Sample No. 5.	Sample No. 10.	Sample No. 16.	Sample No. 19.	Sample No. 20.	Sample No. 25.
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
V. B. Hausknecht, New Jersey	4.26	1.30	2.04	[1]	1.85	0.37	1.39	0.93
	4.17	1.39	1.85	.....	1.85	.28	1.30	.83
Average	4.22	1.35	1.95	.....	1.85	.33	1.35	.88
Emily M. Bresee, Wisconsin	4.90	1.60	1.80	1.80	2.40	.90	2.00	1.60
	4.90	1.60	1.80	1.80	2.40	.90	2.00	1.60
Average	4.90	1.60	1.80	[2] 1.80	2.40	.90	2.00	1.60
J. O. Halverson, Missouri	4.00	1.30	1.30	[3] 1.10	2.10	.40	1.50	1.00
	4.20	1.10	1.40	[3] 1.00	2.00	.40	1.60	[4] 1.10
	4.30			[3] 1.20			1.30	
Average	[5] 4.17	1.20	[5] 1.35	1.10	2.05	.40	1.47	1.05
H. S. Bailey and L. B. Burnett, Washington, D. C	4.14	3.67	3.51	2.89	2.53	.36	1.23	1.03
	4.24	3.82	3.50	3.10	2.53	.36	1.34	.94
Average	4.19	3.75	3.51	3.00	2.53	.36	1.29	.99
Corn Products Refining Co. research laboratory	4.03	1.00	1.10	.55	1.98	.25	1.40	.85
F. D. Fuller, Indiana	4.00	1.00	1.40	.40	1.80	.20	1.40	1.00
	4.05	1.10	1.10	.60	1.90	.20	1.30	1.00
Average	4.03	1.05	1.25	.50	1.85	.20	1.35	1.00
W. J. Jones, jr., Indiana	4.10	1.80	1.20	.90	1.80	.20	1.30	1.30
	3.90	1.80	1.30	.90	1.80	.20	1.30	1.10
Average	4.00	1.80	1.25	.90	1.80	.20	1.30	1.20
General average	4.23	1.73	1.79	1.35	2.07	.39	1.45	1.10

[1] Unable to secure clear filtrate.
[2] Five grams shaken with 200 cc of distilled water; unable to filter 10 grams 200 cc water solution.
[3] Unable to filter; after settled pipette 20 cc off. Add 50 cc redistilled water. Shake, stand, then decant; wash precipitate twice with small amount of water; decant and titrate.
[4] Five grams shaken with 100 cc of distilled water.
[5] Diluted with 100 cc of redistilled water before titration on account of color.

TABLE 2.—*Cooperative results on alkalinity of water extract with Methyl Orange as indicator.*

[Basis, 1 gram.]

Analyst.	Tenth-normal hydrochloric acid.							
	Sample No. 1.	Sample No. 3.	Sample No. 5.	Sample No. 10.	Sample No. 16.	Sample No. 19.	Sample No. 20.	Sample No. 25.
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
V. B. Hausknecht, New Jersey	2.34	2.34	2.86	[1]	1.04	0.26	1.04	1.04
	2.34	2.34	2.86	.....	1.04	.26	1.04	1.04
Average	2.34	2.34	2.86	.....	1.04	.26	1.04	1.04
Emily M. Bresee, Wisconsin	1.60	1.10	2.00	2.80	.90	.30	1.10	1.50
	1.60	1.10	2.00	2.80	.90	.30	1.10	1.50
Average	1.60	1.10	2.00	[2] 2.80	.90	.30	1.10	1.50

[1] Unable to secure clear filtrate.
[2] Five grams shaken with 200 cc of distilled water. Unable to filter 10 grams 200 cc water solution.

TABLE 2.—*Cooperative results on alkalinity of water extract with Methyl Orange as indicator*—Continued.

[Basis, 1 gram.]

Analyst.	Tenth-normal sodium hydroxid.							
	Sample No. 1.	Sample No. 3.	Sample No. 5.	Sample No. 10.	Sample No. 16.	Sample No. 19.	Sample No. 20.	Sample No. 25.
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
J. O. Halverson, Missouri	3.20	2.30	2.70	2.80	.90	.40	1.10	1.70
	3.30	2.60	2.70	2.90	.80	.30	1.40	[1] 2.10
Average	[2] 3.25	2.45	[3] 2.70	[2] 2.85	.85	.35	1.25	1.90
Corn Products Refining Co. research laboratory	.....	1.23	1.03	1.05	.25	.15	.50	1.43
F. D. Fuller, Indiana	1.80	2.10	2.60	1.20	1.00	.30	1.10	1.50
	1.70	2.00	2.30	1.20	1.00	.30	1.15	1.50
	1.80							
	1.70							
Average	1.75	2.05	2.45	1.20	1.00	.30	1.13	1.50
W. J. Jones, jr., Indiana	2.80	2.90	3.10	4.00	1.90	.50	1.50	1.80
	2.60	3.00	3.30	3.20	1.60	.40	1.80	1.90
Average	2.70	2.95	3.20	3.60	1.75	.45	1.65	1.85
General average	2.23	2.09	2.50	2.44	1.03	.32	1.17	1.55

[1] Five grams shaken with 100 cc of distilled water.
[2] Diluted with 100 cc redistilled water on account of color.
[3] Unable to filter. After settled pipette 20 cc off. Add 50 cc of redistilled water, shake, stand, then decant. Wash precipitate twice with small amount of water; decant and titrate.

COMMENTS OF ANALYSTS.

C. S. Cathcart, associate referee: Regarding the acidity, I think sufficient evidence has been presented to show that the acidity generally found in feeds is not due to mineral acids and that care must therefore be used in the form of report on this subject. In view of these facts, I agree with you in favoring the method of reporting the acidity as suggested.

V. B. Hausknecht: Samples were shaken consecutively for 15 minutes by hand with 200 cc of distilled water in a 250 cc flask. Unable to secure clear filtrate from sample No. 10.

Emily M. Bresee: Acidity of all samples except No. 10 was determined in the water extract of the 10 grams shaken in a continuous shaking apparatus for 15 minutes. In the case of No. 10 it was found impossible to filter this extract and another portion of 5 grams was shaken with 200 cc of water. This extract was found easier to filter. The readings given for this sample are twice the amount of the actual readings taken.

J. O. Halverson: Soil shaker used at 100 revolutions per minute with 3½-inch stroke for 15 minutes. Samples did not filter well up to and including Nos. 10, 20, and 25. Sample 10 would not filter. (Extract from sample was decanted, see footnotes Tables 1 and 2.) Acidity on those that filtered slowly, on standing overnight to settle, went up. These results were discarded.

H. C. Humphrey: For water extractions in acidity tests we used frequent hand shaking.

F. D. Fuller: Inasmuch as it has been shown by several workers that phenolphthalein, when used as an indicator to determine the acidity of the water extract of feeds, gives high results in comparison with other indicators, it seems that the results obtained by the method as outlined are of value only in comparing one feed with another. There is no evidence that the results obtained are accurate. The determination of the acidity of a feeding stuff is of very little importance as a criterion in estimating its value for feeding purposes. It will, however, show to what extent fermentation has progressed, and in this regard it may be of some value. Much difficulty was experienced in the method of testing for free inorganic acids, owing to the failure to secure satisfactory end points. It was impossible to titrate to a permanent shade, as the substances held in solution undoubtedly reacted on the alkali and indicator. The method is of value, however, in qualitatively determining the presence of free mineral acids.

W. J. Jones, jr., referee: The water extracts for acidity and alkalinity were secured by shaking in a shaking machine making 35 revolutions per minute for 15 minutes. Difficulty was experienced in filtering the extracts from Samples 3 and 10, and animal charcoal was added, which had but little effect on the rate of filtration and none on the results of the titration. In the titrations for alkalinity difficulty was experienced in securing a distinct end reaction in all solutions, especially those from samples Nos. 1 and 5.

Carl H. McCharles, California:[1] Moisture determinations made in a vacuum oven at 70° C. and under atmospheric pressure reduced by 22 to 25 inch column of mercury; acidity on shaking apparatus takes 300 cc Erlenmeyer flasks.

Determination.	Sample 1.	Sample 3.	Sample 5.	Sample 10.	Sample 16.	Sample 19.	Sample 20.	Sample 25.
Moisture.....per cent..	6.24	6.61	3.04	8.45	8.08	6.09	6.79	10.20
Acidity.....cc..	3.5	.90	1.00	.45	1.75	.20	.75	1.15
Free inorganic acids.....cc..	Alk. 1.20	Alk. 1.30	Alk. [1] 1.00	Alk. [2] 1.65	Alk. .20	Neut.	Alk. .70	Alk. [1] 1.1

[1] Decolorized with animal charcoal, which was found to be neutral in a blank experiment.
[2] 200 cc of 95 per cen alcohol added to mixture after shaking; the whole shaken and filtered and 40 cc titrated; titration corrected for alcohol present by blank experiment.

CONCLUSIONS.

Acidity.—A study of the cooperative results does not show as close agreement as is necessary for absolute deductions, but it is to be noted that on the feed showing the highest acidity all the collaborators with one exception are in close agreement and that on the other samples a majority secure comparable results. In this connection it is also interesting to note that the average results obtained on sample No. 1 are in close agreement with the results reported by the referee on the same feeding stuff in 1910. The results also confirm the statement made in previous reports that there is no apparent definite relation between the amount of protein and the acidity, as is shown by the results on sample No. 5, containing only one-fourth as much protein as No. 3 but showing more acidity.

That steep water in corn gluten feeds increases the acidity is again made evident from the results on Samples 16 and 19, the former of which contains added steep water and has an acidity nearly four times that of No. 19.

In order to make the method applicable to all classes of feeding stuffs, methods for clarifying and promoting more rapid filtration should be available.

Alkalinity.—The results of the titration of the water extract with tenth-normal hydrochloric acid, using Methyl Orange, are at such variance that no deductions are deemed advisable other than that they clearly show that the acidity of ordinary feeding stuffs in general is not due to the presence of free mineral acids. It is possible that under suitable methods for clarifying, solutions may be obtained whereby a distinct end reaction can be secured, but until such time the method will be chiefly valuable as a qualitative test for the presence of free acid.

The results as reported are only added evidence to support the statement made in previous work on this subject; that is, that the acidity of the water extract from feeding stuffs in general is due to the varying amount of complex compounds dissolved and not to added substances.

The determination of the acidity of a feeding stuff, free mineral acids being absent, is of value chiefly as a means of comparison for different materials and in the case of fermentation as a possible measure of the same.

COMPARISON OF THE OFFICIAL (ETHYL ETHER) METHOD AND THE PETROLEUM ETHER METHOD FOR FAT.

The moisture determinations reported in Table 3 are by the official method (5 hours' drying at the temperature of boiling water in hydrogen or vacuo) unless otherwise stated. They present such a wide variation that in order to have the results in comparable form they were reduced to a dry basis. (See Table 4.)

[1] Results sent in too late to be incorporated in table of results.

TABLE 3.—*Cooperative work on official and petroleum-ether methods for the determination of fat.*

Analyst.	Sample No. 1.			Sample No. 3.			Sample No. 5.			Sample No. 10.		
	Moisture.	Ether extract.	Petroleum ether extract.	Moisture.	Ether extract.	Petroleum ether extract.	Moisture.	Ether extract.	Petroleum ether extract.	Moisture.	Ether extract.	Petroleum ether extract.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Victor B. Hausknecht, New Jersey.	6.96	12.09	10.78	7.26	10.69	10.84	4.28	3.14	2.90	8.92	9.32	9.02
	7.00	12.12	10.84	7.38	10.60	10.98	4.42	3.08	2.90	8.98	9.21	8.90
Average	6.98	12.11	10.81	7.32	10.65	10.91	4.35	3.11	2.90	8.95	9.27	8.96
Emily M. Bresee, Wisconsin.[1]	[2]10.23	11.47	10.94	[2]7.70	11.29	10.12	[2]7.73	3.16	2.89	[2]9.61	8.35	8.83
	[2]10.26	11.39	10.86	[2]7.72	11.20	10.14	[2]7.70	3.12	2.94	[2]9.62	8.30	8.94
	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	8.31	8.90
Average	10.25	11.43	10.90	7.71	11.25	10.13	7.72	3.14	2.92	9.62	8.32	8.89
H. J. White, Maryland	[3]7.02	[4]17.70	[3]9.09	[3]4.11	[4]11.52	[3]5.34	2.02	[4]4.89	[3]1.96	5.87	[4]10.16	[3]6.00
	[3]7.14	[4]18.10	[3]9.02	[3]4.10	[4]11.53	[3]5.28	2.04	[4]4.71	[3]1.94	5.88	[4]9.99	[3]5.95
Average	7.08	17.90	9.06	4.11	11.53	5.31	2.03	4.80	1.95	5.88	10.08	5.98
J. O. Halverson, Missouri	6.50	13.50	10.83	8.46	11.12	[3]8.58	3.54	3.71	2.80	9.78	8.50	9.61
	6.33	13.02	9.75	8.57	10.68	[3]8.44	3.73	3.66	2.76	10.00	8.94	9.51
	.....	.....	[3]5.76	.....	[5]10.75	[3]7.07	.....	.....	3.03	.....	.....	8.93
	.....	.....	[6]9.83	.....	.....	[7]7.92	.....	.....	.....	.....	.....	[8]8.16
Average	6.42	13.27	10.14	8.52	10.85	8.00	3.64	3.69	2.86	9.89	8.72	9.35
W. Levin, Texas	6.28	11.65	10.95	6.88	11.25	9.75	3.27	2.95	2.54	7.50	9.50	8.89
H. S. Bailey and L. B. Burnett, Washington, D. C.	5.45	13.40	11.17	5.43	11.00	10.18	2.05	3.43	3.02	6.95	9.70	9.33
	5.33	13.31	11.42	5.47	[9]10.67	.....	2.10	[9]3.37	3.02	6.90	[9]9.25	.....
Average	5.39	13.36	11.30	5.45	10.84	10.18	2.08	3.40	3.02	6.93	9.48	9.33
Corn Products Refining Co., Research Laboratory.	6.89	10.88	10.80	7.37	10.83	10.03	4.15	3.17	2.74	9.24	9.21	8.95
	6.83	11.02	10.84	7.38	10.69	9.97	4.12	3.16	2.75	9.30	9.39	8.81
Average	6.86	10.95	10.82	7.38	10.76	10.00	4.14	3.17	2.75	9.27	9.30	8.88
C. Cutler, Indiana	7.24	12.26	11.02	5.93	10.57	10.33	3.61	3.03	2.70	7.70	9.18	9.17
	7.23	12.30	11.05	5.90	10.66	9.99	3.64	3.06	2.71	7.71	8.98	9.21
	7.14	12.26	.....	6.25	10.73	.....	3.85	3.15	.....	7.80	9.13	.....
	6.91	12.25	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average	7.13	12.27	11.04	6.03	10.65	10.16	3.70	3.08	2.71	7.74	9.10	9.19
F. D. Fuller, Indiana	7.44	12.79	11.14	6.22	10.74	10.47	4.18	3.27	2.84	7.60	9.37	9.15
	7.52	12.53	11.10	5.99	10.94	10.50	3.80	3.30	2.81	7.51	9.35	9.23
Average	7.48	12.66	11.12	6.11	10.84	10.49	3.99	3.29	2.83	7.56	9.36	9.19
W. J. Jones, jr., Indiana	7.41	12.16	11.02	6.36	10.86	10.07	4.07	3.15	2.89	8.25	9.36	9.20
	7.30	11.96	11.03	6.38	10.69	10.27	4.03	3.19	2.82	8.09	9.36	9.29
	7.38	12.07	.....	6.43	10.86	.....	4.09	3.32	.....	7.69	9.07	.....
	7.36	11.98	.....	6.32	10.90	.....	4.05	3.36	.....	7.59	9.09	.....
	.....	.....	.....	.....	.....	.....	.....	.....	.....	7.55	9.15	.....
	.....	.....	.....	.....	.....	.....	.....	.....	.....	7.70	9.29	.....
Average	7.36	12.04	11.03	6.37	10.83	10.17	4.06	3.26	2.86	7.61	9.22	9.25
Mean of all determinations reported	7.18	12.70	10.44	6.53	10.90	9.31	3.93	3.38	2.75	8.07	9.18	8.76
Mean of determinations with omissions	6.87	12.21	10.96	6.67	10.84	10.26	3.55	3.24	2.84	8.14	9.10	9.10

[1] Extractions made in Caldwell extractors.
[2] Moisture determined in air bath at 110° C. Results not considered in the averages used for comparing methods.
[3] Results not considered in the averages used for comparing methods.
[4] Ether containing alcohol used for extraction. Results not considered in the averages used for comparing methods.
[5] Moisture removed by drying 6½ hours in electric oven at 100° C.
[6] Additional rapid extraction for 3 hours gave 0.0235 grams oil, making total extraction in 6 hours, 11.01 per cent.
[7] Additional rapid extraction for 3 hours gave 0.0513 grams oil, making total extraction in 6 hours, 10.49 per cent.
[8] Additional rapid extraction for 3 hours gave 0.0119 grams oil, making total extraction in 6 hours, 8.75 per cent.
[9] Extraction made in a Johnson continuous extractor.

TABLE 3.—*Cooperative work on official and petroleum-ether methods for the determination of fat*—Continued.

Analyst.	Sample No. 16.			Sample No. 19.			Sample No. 20.			Sample No. 25.		
	Moisture.	Ether extract.	Petroleum ether extract.	Moisture.	Ether extract.	Petroleum ether extract.	Moisture.	Ether extract.	Petroleum ether extract.	Moisture.	Ether extract.	Petroleum ether extract.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Victor B. Hausknecht, New Jersey.	8.89	5.32	4.98	6.47	5.57	5.12	7.50	5.23	4.58	10.82	5.01	4.30
	8.84	5.23	4.78	6.52	5.41	4.95	7.43	5.28	4.62	10.90	5.04	4.23
Average	8.87	5.28	4.88	6.50	5.49	5.04	7.47	5.26	4.60	10.86	5.03	4.27
Emily M. Bresee, Wisconsin. [1]	[2] 9.90	5.37	4.88	[2] 6.90	4.46	5.02	[2] 7.65	4.74	4.22	[2] 10.95	5.48	4.08
	[2] 9.91	5.41	4.76	[2] 6.93	4.42	4.98	[2] 7.68	4.67	4.34	[2] 10.98	5.44	3.93
	.....	.....	.....	.....	4.44	4.90	.....	.....	.....	.....	.....	.....
Average	9.91	5.39	4.82	6.92	4.44	4.97	7.67	4.71	4.28	10.97	5.46	4.01
H. J. White, Maryland	[3] 6.37	6.08	4.75	[3] 3.21	4.89	[3] 1.63	[3] 4.58	[4] 6.47	[3] 1.69	[4] 7.26	.....	.....
	[3] 6.36	6.16	4.79	[3] 3.16	5.00	[3] 2.33	[3] 4.58	[4] 6.54	[3] 1.60	[4] 7.25	.....	.....
Average	6.37	6.12	4.77	3.19	4.95	1.98	4.58	6.51	1.65	7.26	.....	.....
J. O. Halverson, Missouri	8.91	6.01	4.92	6.61	5.40	5.04	7.61	5.91	4.49	11.56	5.14	4.30
	8.79	5.91	4.86	7.11	5.30	5.05	7.74	5.63	4.53	11.23	5.14	3.72
	.....	.....	4.86	.....	.....	5.11	.....	.....	4.32	.....	.....	4.16
Average	8.85	5.96	4.88	6.86	5.35	5.07	7.68	5.77	4.45	11.40	5.14	4.06
W. Levin, Texas	7.25	5.50	4.80	5.05	5.30	4.42	6.28	6.02	4.86	9.52	4.79	3.45
H. S. Bailey and L. B. Burnett, Washington, D. C.	7.20	5.42	5.48	4.72	5.40	5.72	6.00	5.42	4.80	9.00	5.10	4.50
	7.22	[5] 5.37	.....	4.90	5.38	5.45	6.00	[5] 5.00	.....	9.00	5.25	4.35
	.....	.....	.....	.....	[5] 5.45	.....	.....	.....	.....	.....	.....	.....
	.....	.....	.....	.....	[5] 5.30	.....	.....	.....	.....	.....	.....	.....
Average	7.21	5.40	5.48	4.81	5.38	5.59	6.00	5.21	4.80	9.00	5.18	4.43
Corn Products Refining Co., Research Laboratory.	9.07	5.09	4.85	6.56	4.61	5.04	7.65	4.46	4.41	10.97	4.61	4.26
	9.03	4.95	4.86	6.58	4.76	4.99	7.59	4.40	4.45	10.95	4.51	4.23
Average	9.05	5.02	4.85	6.57	4.69	5.01	7.62	4.43	4.43	10.96	4.56	4.24
C. Cutler, Indiana	8.08	5.15	5.00	5.00	4.75	5.00	6.43	4.93	4.35	9.46	4.81	3.96
	8.03	5.12	4.96	5.43	4.72	5.04	6.46	4.84	4.37	9.44	4.79	3.86
	8.07	5.26	.....	5.34	4.78	.....	6.69	4.80	.....	9.57	4.81	.....
Average	8.06	5.18	4.98	5.26	4.75	5.02	6.53	4.86	4.36	9.49	4.80	3.91
F. D. Fuller, Indiana	8.47	5.34	5.13	5.58	5.01	5.15	7.07	5.03	4.34	9.92	4.93	4.07
	8.36	5.32	5.09	5.26	4.96	5.13	6.96	4.98	4.33	9.74	4.89	3.98
Average	8.42	5.33	5.11	5.42	4.99	5.14	7.02	5.01	4.34	9.83	4.91	4.03
W. J. Jones, jr., Indiana	8.27	5.33	4.82	5.62	4.99	5.15	7.09	4.94	4.46	9.91	4.91	4.10
	7.95	5.43	4.98	5.62	4.95	5.17	6.87	5.00	4.49	10.07	4.97	4.06
	8.13	5.46	.....	5.09	4.83	.....	6.09	4.63	.....	9.15	4.98	.....
	8.00	5.48	.....	5.15	4.77	.....	6.32	4.77	.....	9.27	4.92	.....
	7.79	5.29	.....	5.63	5.04	.....	6.17	5.01	.....	9.43	4.88	.....
	7.83	5.31	.....	5.39	4.96	.....	6.13	4.92	.....	9.29	4.96	.....
	7.68	5.46	.....	5.05	4.95	.....	6.52	5.13	.....	9.89	5.18	.....
	7.81	5.44	.....	5.10	4.97	.....	6.46	5.19	.....	9.77	5.16	.....
Average	7.93	5.40	4.90	5.33	4.93	5.16	6.46	4.95	4.48	9.60	5.00	4.08
Mean of all determinations reported	8.16	5.43	4.92	5.54	4.99	4.78	6.68	5.15	4.17	9.82	4.99	4.09
Mean of determinations with omissions	8.16	5.43	4.92	5.63	4.99	5.07	6.78	5.04	4.47	9.95	4.99	4.09

[1] Extractions made in Caldwell extractors.
[2] Moisture determined in air bath at 110° C. Results not considered in the averages used for comparing methods.
[3] Results not considered in the averages used for comparing methods.
[4] Ether containing alcohol used for extraction. Results not considered in the averages used for comparing methods.
[5] Extraction made in a Johnson continuous extractor.

TABLE 4.—*Cooperative results on official and petroleum-ether methods for the determination of fat.*

[Average results calculated to dry matter.]

Analyst.	Sample No. 1.		Sample No. 3.		Sample No. 5.		Sample No. 10.	
	Ether extract.	Petroleum ether extract.	Ether extract.	Petroleum ether extract.	Ether extract.	Petroleum ether extract.	Ether extract.	Petroleum ether extract.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
V. B. Hausknecht, New Jersey	13.02	11.62	11.49	11.77	3.25	3.03	10.18	9.84
Emily M. Bresee, Wisconsin	12.73	12.14	12.19	10.98	3.40	3.16	9.21	9.84
H. J. White, Maryland	[1] 19.26	[1] 9.75	12.02	[1] 5.54	[1] 4.90	[1] 1.99	[1] 10.71	[1] 6.35
J. O. Halverson, Missouri	14.18	10.83	11.86	[1] 8.75	3.83	2.97	9.68	10.37
W. Levin, Texas	12.43	11.68	12.08	10.47	3.05	2.63	10.27	9.61
H. S. Bailey and L. B. Burnett, Washington, D. C.	14.12	11.94	11.46	10.77	3.47	3.08	10.19	10.02
Corn Products Refining Co., Research Laboratory	11.76	11.62	11.62	10.80	3.31	2.86	10.25	9.79
C. Cutler, Indiana	13.21	11.89	11.33	10.81	3.20	2.81	9.86	9.96
F. D. Fuller, Indiana	13.68	12.02	11.54	11.17	3.42	2.95	10.13	9.94
W. J. Jones, jr., Indiana	13.00	11.91	11.57	10.86	3.40	2.98	9.98	10.01
Mean of all determinations reported	13.74	11.54	11.72	10.19	3.52	2.85	10.05	9.57
Mean of determinations with omissions	13.13	11.74	11.72	10.95	3.37	2.94	10.07	9.93

Analyst.	Sample No. 16.		Sample No. 19.		Sample No. 20.		Sample No. 25.	
	Ether extract.	Petroleum ether extract.	Ether extract.	Petroleum ether extract.	Ether extract.	Petroleum ether extract.	Ether extract.	Petroleum ether extract.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
V. B. Hausknecht, New Jersey	5.79	5.36	5.87	5.39	5.68	4.97	5.62	4.79
Emily M. Bresee, Wisconsin	5.98	5.35	4.77	5.34	5.10	4.64	6.13	4.50
H. J. White, Maryland	6.54	5.09	5.11	[1] 2.05	[1] 6.82	[1] 1.74	.....	.....
J. O. Halverson, Missouri	6.54	5.35	5.75	5.44	6.25	4.82	5.80	4.58
W. Levin, Texas	5.93	5.18	5.58	4.66	6.42	5.19	5.29	3.81
H. S. Bailey and L. B. Burnett, Washington, D. C.	5.82	5.91	5.65	5.87	5.54	5.11	5.69	4.87
Corn Products Refining Co., Research Laboratory	5.52	5.33	5.02	5.36	4.80	4.80	5.12	4.76
C. Cutler, Indiana	5.63	5.42	5.01	5.30	5.20	4.66	5.30	4.32
F. D. Fuller, Indiana	5.82	5.58	5.28	5.43	5.39	4.67	5.45	4.47
W. J. Jones, jr., Indiana	5.87	5.32	5.21	5.45	5.29	4.79	5.53	4.51
Mean of all determinations reported	5.94	5.39	5.33	5.03	5.65	4.54	5.55	4.51
Mean of determinations with omissions	5.94	5.39	5.33	5.36	5.52	4.85	5.55	4.51

[1] Results not considered in the averages used for comparing methods.

COMMENTS OF ANALYSTS.

C. S. Cathcart, associate referee: The "petroleum ether extract" is lower than the "ether extract" in every case, excepting for Sample 3. As this condition is to be expected, I do not think it would be a wise step to recommend this method as even a provisional method and leave the ether method in force. The results obtained in the inspection work would depend upon the method used, and in those cases where the analyses are checked by another chemist, and the same method not used, there would be a discussion regarding the results.

Emily M. Bresee: The fat extractions were made in Caldwell extractors in place of Soxhlet extractors recommended in directions.

H. J. White (August 23): The ether used in this determination was as it comes from the manufacturers, containing 3.25 per cent alcohol.

H. O. Halverson: Samples Nos. 1, 3, and 10, high in oil, when extracted 3 hours by the petroleum ether method did not check well. Another, a third determination of all was run. Nos. 1, 3, and 10 did not check, results being lower. A fourth determination was run, using more heat, extraction being more rapid. Results given on a separate sheet indicate that they were still low. These same cones from the fourth determination were then vigorously extracted for 3 additional hours, running 6 hours in all, giving increased oil, Nos. 1, 3, and 10, respectively, 23.5 mg, 51.3 mg, and 11.9 mg, giving total oil for the fourth determination as follows: 0.2201 gram, 0.2097 gram, and 0.1750 gram.

This indicates that the rate of extraction should also be specified. Three hours' extraction may not extract all the oil in feeds high in oil.

G. S. Fraps: Mr. Levin did not get good results by the petroleum ether method.

H. S. Bailey: Relative to the extractions with petroleum ether, I was a little dubious at first as to whether the 3 hours was sufficient time to get the complete extraction, and therefore made one analysis of Sample 1 in a Soxhlet having a stopcock at the side of the siphon tube so that samples could be taken out each time before the liquid siphoned over. I find that 25 cc drawn off the fifth time the extractor filled up yielded on evaporation only 1 mg of extract. The determination had been running then for about 3 hours. In the case of Sample 1, in which the petroleum extract is nearly 2 per cent lower than the ether extract, it is evident that practically all the petroleum ether soluble substances have been extracted at the end of 3 hours.

W. J. Jones, jr., referee: Much difficulty was encountered in securing concordant results on moisture on different days. All determinations were by the official methods in hydrogen. All results reported are duplicates on separate sets of the samples. Difficulty was experienced in securing continuous extraction with the petroleum ether and after the extraction had proceeded for 1½ to 2 hours it was found necessary to add a fresh amount of petroleum ether in order to continue the extraction. It was also found impossible to distill the total amount of the petroleum ether (approximately 25 cc) into the extractor on completion of the extraction, and this was evaporated in the open.

Difficulty was experienced in drying the extract to constant weight and while the ether extracts are readily dried by heating at the temperature of boiling water for 1½ hours it was found necessary to dry the petroleum ether extracts 4 hours.

COMPARISON OF THE COOPERATIVE RESULTS.

The results on moisture are so variable that it is thought advisable to compare the determinations on a moisture-free basis. In this comparison some of the results are omitted, for the reason that variations from the official methods were used in obtaining them, or they differ so much from the average as to indicate some unknown cause of variation.

Taken as a whole the results by the petroleum ether method show closer agreement among the majority of the analysts on every sample than by the official method, but in all samples a higher average result, varying from 0.18 per cent in sample No. 10 to 1.39 per cent in sample No. 1, with a maximum variation from the average by the various analysts of 0.58 per cent in sample No. 16 to 3.35 per cent in sample No. 1, are secured by the official method.

The majority of the analysts secured an excess of fat in all samples by the official method, with the exception of No. 19, in which six secured an average excess of 0.30 per cent with a range of 0.15 to 0.57 per cent by the petroleum ether method.

Four analysts secured an excess by the petroleum ether method in sample No. 10 and one in each of samples Nos. 1 and 16.

ADDITIONAL WORK BY THE REFEREE.

Having found that it was necessary to dry the petroleum ether extracts longer than the ethyl ether extracts to secure constant weights, it was decided to dry a series 2, 4, and 6 hours to determine if possible the maximum period required. The flasks were desiccated and weighed at the end of each period. The results given in Table 5 show that in all the extracts except those from samples Nos. 10 and 16 an appreciable loss occurred between the 2 and 4 hour period, but that in all cases the drying was

complete at 4 hours, as shown by lack of appreciable loss during the third 2-hour period.

It was also deemed advisable to test the completeness of the extraction. For this purpose the residues in the capsules from the 3-hour extraction were extracted 13 additional hours, making the total period of extraction 16 hours. The results given in the second part of Table 5 show that an increase from the second extraction resulted in every case and that in five samples of the eight the increase was appreciable.

TABLE 5.—*Effect of length of time of drying extract and extracting with petroleum ether.*

Sample No.	Three hours' extraction.					Additional extraction for 13 hours.			
	Dried 2 hours 100° C.	Dried 4 hours 100° C.	Dried 6 hours 100° C.	Gain or loss, 2 to 4 hours' drying.	Gain or loss, 4 to 6 hours' drying.	Dried 4 hours 100° C.	Dried 6 hours 100° C.	Gain or loss, 4 to 6 hours' drying.	Gain from second extraction.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	11.45	11.02	11.08	—0.43	+0.06	11.35	11.27	—0.08	+0.33
	11.44	11.03	11.00	— .41	— .03	11.38	11.30	— .08	+ .35
	11.45	11.03	11.04	— .42	+ .015	11.37	11.29	— .08	+ .34
3	10.62	10.07	10.04	— .55	— .03	10.39	10.38	— .01	+ .32
	10.85	10.27	10.24	— .57	— .03	10.58	10.52	— .06	+ .31
	10.74	10.17	10.14	— .56	— .03	10.49	10.45	— .04	+ .32
5	3.30	2.89	2.78	— .41	— .11	2.97	2.90	— .07	+ .08
	3.20	2.82	2.81	— .38	— .01	3.00	2.91	— .09	+ .18
	3.25	2.86	2.80	— .40	— .06	2.99	2.91	— .08	+ .13
10	9.12	9.20	9.22	+ .08	+ .02	9.58	9.57	— .01	+ .38
	9.14	9.29	9.42	+ .15	+ .13	9.62	9.58	— .04	+ .33
	9.13	9.25	9.32	+ .12	+ .08	9.60	9.58	— .02	+ .36
16	4.78	4.82	4.75	+ .04	— .07	4.95	4.98	+ .03	+ .13
	4.96	4.98	5.01	+ .02	+ .03	5.05	5.13	+ .08	+ .07
	4.87	4.90	4.88	+ .03	— .02	5.00	5.06	+ .06	+ .10
19	5.12	5.15	5.06	+ .03	— .09	5.12	5.14	+ .02	— .03
	5.44	5.17	5.14	— .27	— .03	5.18	5.19	+ .01	+ .01
	5.28	5.16	5.10	— .12	— .06	5.15	5.17	+ .02	— .01
20	4.67	4.46	4.49	— .21	+ .03	4.78	4.80	+ .02	+ .32
	4.79	4.49	4.55	— .30	+ .06	4.84	4.88	+ .04	+ .35
	4.73	4.48	4.52	— .25	+ .04	4.81	4.84	+ .03	+ .33
25	4.71	4.10	4.05	— .61	— .05	4.65	4.66	+ .01	+ .55
	4.73	4.06	4.05	— .67	— .01	4.62	4.62	.....	+ .56
	4.72	4.08	4.05	— .64	— .03	4.64	4.64	.....	+ .56

The variation in the composition of petroleum products apparently having the same general physical characteristics but from different sources being well known, it was considered advisable if possible to make determinations with different petroleum ethers. Unfortunately, only two could be secured, but some results were also obtained for comparison by using that portion distilling below 65° C. of the petroleum ether used in securing the results in Tables 3 and 4.

The petroleum ether used in securing the results given in the second column gave 86 per cent distillate below 65° C. and all distilled at 72½° C. The distillation beginning at 31° C., 100 cc gave a residue of 0.7 mg. The petroleum ether used in securing results given in the third column was the distillate below 65° C. of the ether used in the second column. The pentane, secured through the courtesy of Mr. Burton, superintendent of the Standard Oil Co., Whiting, Ind., was made from Kansas crude

oil and stated to give a distillate of 98 per cent below 65° C. On distillation 99 per cent came off below 65° C., the distillation beginning at 35° C. One hundred cubic centimeters gave a residue of 3.5 mg. The ether used to secure the results given in the last column was the same as used in the second, the samples being dried for five hours in hydrogen at the temperature of boiling water before extraction.

No very appreciable difference was noted in the comparison of the three petroleum ethers, although in the case of all but one sample those having the lower distilling point give a decrease in the amount extracted. In the case of all but one sample the amount of extract from the moisture-free samples is less than that from those extracted without removal of the moisture. This is especially noticeable in the case of samples Nos. 19 and 20, where the difference is very appreciable. Unfortunately, time was not available to study the reason for the marked loss in these two samples, though it is hoped to take up the subject again.

The results of the investigation appear in Table 6:

TABLE 6.—*Comparison of petroleum ether extractions.*

[4 hours drying.]

Sample No.	Petroleum ether boiling below 65° C.	Petroleum ether distilling below 65° C.	Pentane.	Petroleum ether boiling below 65° C. with dried sample.[1]
	Per cent extract.	*Per cent extract.*	*Per cent extract.*	*Per cent extract.*
1	11.02	10.79	10.99	10.70
	11.03	10.90	11.09	10.67
	11.03	10.85	11.04	10.69
3	10.07	10.15	10.34	10.19
	10.27	10.20	9.29	10.31
	10.17	10.18	9.82	10.25
5	2.89	2.75	2.85	2.61
	2.82	2.63	2.87	2.64
	2.86	2.69	2.86	2.63
10	9.20	9.09	9.31	9.12
	9.29	9.27	9.07	9.10
	9.25	9.18	9.19	9.11
16	4.82	5.02	5.06	4.87
	4.98	5.06	5.06	4.88
	4.90	5.04	5 06	4.88
19	5.15	5.06	5.14	2.95
	5.17	4.96	5.10	2.85
	.....	.....	.....	2.39
	.....	.....	.....	2.33
	5.16	5.01	5.12	2.63
20	4.46	4.41	4.53	3.94
	4.49	4.30	4.33	3.94
	.....	.....	.....	3.76
	.....	.....	.....	3.82
	4.48	4.36	4.43	3.87
25	4.10	3.98	3.90	3.85
	4.06	3.90	4.04	3.78
	4.08	3.94	3.97	3.82

[1] Determinations by F. D. Fuller.

While it is generally known that the presence of moisture either in the ethyl ether used for extracting or in the sample materially affects the amount of extract and that the presence of alcohol produces a similar effect, the increased utilization of by-products from different sources as feeding stuffs in the last five years tends to increase the chances for error due to failure to use absolute ether in the extraction. That the importance of so doing is being overlooked in some cases has been made evident in the Indiana inspection, since it has been found that some chemists were using Squibbs ether containing alcohol, that others were extracting the samples without drying, that in many cases the ether was used as received from the manufacturer without further testing or purification, and that in still others the moisture was expelled at a higher temperature than 100° C. without the use of hydrogen or vacuum.

The variations mentioned have caused such differences in results and have in a number of cases caused many manufacturers such serious inconvenience due to the

making of too high guarantees for crude fat that the following work was planned with a view to again calling attention to the necessity of following official methods to the letter if comparable results were desired. Table 7 contains the results.

TABLE 7.—*Effect of extracting with different ethers and with and without previous drying of sample.*

Sample No.	Moisture at 100° C. in hydrogen.	Loss on drying 5 hours at 110° C. in air bath.	Official absolute ether.[1] Extract.	Ether distilled from metallic sodium as received from manufacturer. Extract.[2]	Official absolute ether without drying sample. Extract.	Official absolute ether after exposing dried sample to air 12 hours. Extract.	Official absolute ether, sample dried at 110° C. in air bath. Extract.	Squibbs ether for anesthesia after drying sample. Extract.	Squibbs ether for anesthesia without drying sample. Extract.[2]
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	.....	12.87	12.16	12.41	13.50	[3] 15.12	7.89	17.49	18.08
	.....	12.69	11.96	12.16	13.66	15.10	8.05	18.21	17.66
	7.36	12.78	12.06	12.28	13.58	15.11	7.97	17.85	17.87
3	.....	7.45	10.86	10.87	10.86	10.46	10.35	11.00	11.40
	.....	7.39	10.69	10.73	11.12	10.48	10.35	11.00	11.45
	6.37	7.42	10.78	10.80	10.99	10.47	10.35	11.00	11.43
5	.....	9.00	3.15	3.38	3.39	4.22	2.73	3.76	5.10
	.....	8.83	3.19	3.41	3.45	4.33	2.57	4.10	5.35
	4.06	8.92	3.17	3.40	3.42	4.28	2.65	3.93	5.23
10	.....	9.21	9.36	9.34	9.39	9.07	3.92	9.38	9.60
	.....	8.89	9.36	9.43	9.38	9.09	4.06	9.38	9.57
	.....	9.05	.....	.....	.....	.....	4.66	.....	.....
	.....	9.37	.....	.....	.....	.....	4.22	.....	.....
	7.61	9.13	9.36	9.39	9.39	9.08	4.22	9.38	9.59
16	.....	9.99	5.33	5.45	5.48	5.46	3.53	6.59	6.63
	.....	9.47	5.43	5.48	5.44	5.68	3.78	6.71	6.60
	7.93	9.73	5.38	5.47	5.46	5.57	3.66	6.65	6.62
19	.....	6.99	4.99	5.30	5.64	4.83	1.98	6.09	5.68
	.....	7.01	4.95	5.30	5.39 5.47	4.77	1.99	5.96	5.62
	5.33	7.00	4.97	5.30	5.43	4.80	1.99	6.03	5.65
20	.....	8.82	4.94	5.18	5.09	4 63	2.72	6.08	6.09
	.....	8.84	5.00	5.14	.....	4.77	2.78	6.22	6.42
	6.46	8.83	4.97	5.16	5.09	4.70	2.75	6.15	6.26
25	.....	11.76	4.91	5.00	5.11	4.98	3.41	5.33	5.48
	.....	11.72	4.97	5.02	5.15	4.92	3.47	5.57	5.43
	9.60	11.74	4.94	5.01	5.13	4.95	3.44	5.45	5.46

[1] C. P. ether distilled over metallic sodium; before being used it is desiccated over fresh metallic sodium for 2 or 3 weeks and redistilled from fresh portions of metallic sodium.
[2] Determinations by F. D. Fuller.
[3] It is interesting to note that this sample took up 6.24 per cent of moisture as compared with 7.13 per cent loss on drying.

The results speak for themselves and need no comment, but attention is called to the results in the last two columns, in which it is shown that on most of the samples the presence of water materially increases the amount of extract over that obtained from ether containing alcohol.

CONCLUSIONS.

The results indicate that in all classes of feeding stuffs the official methods for estimating crude fat may be expected to give appreciably higher results than the petroleum ether method.

That the official method is to be preferred from the standpoint of ease in extraction, more rapid and perfect drying of the extract, and less objectionable reagent to handle.

That the petroleum ether method has the following points of advantage over the official: The matter extracted more nearly represents true fat; previous drying of the sample is omitted (from Table 6 it would appear that the assumption that the moisture content does not affect the extraction may be in error at least in certain products); the time required for complete extraction is materially reduced; the results are not susceptible to as many working errors as the official; the cost of the extracting reagent is materially less; and the method seems to give more concordant results in the hands of different analysts.

That results by the official method are appreciably affected by variations in the composition of the extracting reagent or condition of the sample, and in order to secure accurate and comparable results between analysts the sample must be moisture-free, must not be dried above 100° C., and absolute ether must be used for the extraction.

Recommendations.

It is recommended—

(1) That the method for determining acidity proposed by the previous referee be adopted as a provisional method.

(2) That the acidity of feeding stuffs be reported in terms of tenth-normal sodium hydroxid required for neutralization.

(3) That the petroleum ether method for the determination of fat be a subject of further study, especially in regard to the comparison of the effects of petroleum ethers from different crude petroleums, the rate of extraction, the length of time of drying the extract, and the effect of moisture in the sample.

(4) That in the petroleum ether method the word "distilling" be substituted for the word "boiling," making the method read "Extract 2 to 5 grams of the sample, without previous drying for three hours in a Soxhlet apparatus with petroleum ether distilling below 65° C."

(5) That in view of the nature of the problems the questions of the "proper factor for the conversion of nitrogen into protein" and "study of acidity in feeding stuffs with special reference to eliminating the error due to proteins" be referred to a special committee for continuous investigation.

The referee regrets that the advanced date of the meeting and short notice received have absolutely prevented the presentation of a résumé of any recent work on the subjects assigned.

REPORT ON SUGAR AND MOLASSES.

By Wm. E. Cross, *Referee.*

The work of the referee on sugar and molasses during the past year has been along the lines recommended by the association at its last meeting, so far as that was deemed advisable. In order that the work might not prove too cumbersome to invite cooperation, it was thought best to leave the question of the factor for commercial glucose determination, and the composition of basic lead acetate, for some future year.

One first molasses, one blackstrap, and one sugar were sent out with the following instructions:

Instructions.

MOISTURE IN SUGARS (1 SAMPLE).

(1) Determine moisture content of sugar sample by the following method: Weigh out 20 grams of sample into a tared flask and add about 20 grams of water. (An ordinary 100 cc sugar flask with narrow neck is suitable.) Stopper the flask to prevent evaporation and dissolve the sugar completely by shaking. The total solids value of the solution is obtained by the Abbé refractometer and temperature correction applied. Per cent of moisture in sugar $= \frac{2000 - XY}{20}$. X=per cent total solids of solution; Y=weight (grams) of sugar (20 grams) water.

(2) Determine moisture in sugar by the following method: Weigh out 20 grams of the sugar, dissolve in a 100 cc flask, and make up the solution to the mark. Read off the refractive value of this solution by means of the immersion refractometer (temperature must be constant and carefully noted). From this value the moisture content of the sugar is obtained by reference to the tables.[1]

(3) Determine moisture in sugar sample by the official method (Bureau of Chemistry Bul. 107, Rev., p. 64).

MOISTURE IN MOLASSES (2 SAMPLES).

(1) Determine moisture in each of the samples by heating with sand at temperature of boiling water for 10 hours (Bureau of Chemistry Bul. 107, Rev., p. 65).

(2) Determine moisture in each sample by the Abbé refractometer (diluting with sugar solution if necessary).

(3) Determine moisture in each sample by the immersion refractometer.

POLARIZATION OF MOLASSES (2 SAMPLES.)

(1) Determine the true sucrose content of the samples by the official method (Bureau of Chemistry Bul. 107, Rev., p. 40).

(2) Determine the true sucrose content of the samples by the following modification of the Clerget method.[1] Dissolve normal weight of molasses and make up to 200 cc. Treat a convenient quantity with excess of dry lead subacetate, sufficient to insure a very good clarification, and filter. To the filtrate add not quite sufficient dry finely powdered oxalic acid (anhydrous) to precipitate the lead present in solution, and refilter the mixture. Make the single polarization and the Clerget determination with this solution.

(3) Determine true sucrose content of the samples by the following direct method:[2] Dissolve normal weight of molasses and make up to 100 cc. Transfer 50 cc of the solution to another 100 cc flask, where 6.3 cc of sodium hydroxid solution (36° Baumé) and 7.5 cc of hydrogen peroxid (30 per cent by weight, 100 per cent by volume) are added. Careful cooling is necessary to prevent a too violent effervescence (ether from a dropping funnel may be used to advantage to prevent excessive foaming). Cooling in water or ice is helpful in moderating the somewhat vigorous reaction. After effervescence has almost stopped, immerse the flask in a bath at 55° C. for 20 minutes. Cool the liquid, make slightly acid with acetic acid, and make up to mark. After clarification with dry lead subacetate, filter and polarize the solution. The reading multiplied by 2 gives the percentage of true sucrose in the molasses.

Please report all temperatures and concentrations used.

It is desirable that the cooperative work should be carried out as soon as possible after the receipt of samples, as deterioration and fermentation are likely to take place if the samples are allowed to stand any length of time.

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[1] Stanek, International Sugar Journal, 1911, *13:* 90.

[2] Cross and Taggart, International Sugar Journal, 1912, vol. *14:* La. Agr. Exper. Sta. Bul. 135.

Results of Cooperative Work.

Table 1.—*Comparative results on moisture in sugar and molasses.*

Analyst.	Moisture in sugar.			Moisture in first molasses.			Moisture in blackstrap.		
	Abbé.	Immersion.	Official method.	Abbé.	Immersion.	Official method.	Abbé.	Immersion.	Official method.
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
W. E. Cross, New Orleans, La.	0.79	0.73	1.08	[1] 24.30	[2] 24.04	24.38	[1] 20.43	[2] 19.18	.....
								[3] 18.65	.....
W. G. Taggart, New Orleans, La.	.92	.78	.....	[1] 24.31	[2] 24.08	.....	[1] 20.58	[3] 19.08	.....
								[4] 19.18	.....
W. J. McGee, New Orleans, La.	.....	1.35	1.25	24.21	.....	24.8	20.76	[5] 20.9	22.5
E. H. Grant, New Orleans, La.	.72	1.28	1.17	24.55	.....	25.95	20.53	[6] 19.45	23.85
H. E. Z. Perkins, New Orleans, La.	.....	.....	.....	.....	.....	.....	.....	.....	.....
Geo. P. Meade, Gramercy, La.	1.36	.....	1.06	[1] 24.69	.....	.....	20.79	.....	.....
G. B. Williamson, Gramercy, La.	.....	.....	.....	.....	.....	26.11	.....	.....	23.41
S. F. Sherwood, Washington, D. C.	.99	.73	[7] .95	23.91	23.73	[8] 25.50	19.89	.....	[9] 23.90
E. J. Hough, Washington, D. C.	.99	.82	.....	23.96	24.50	26.02	20.00	.....	.....
Average	.96	.95	1.10	24.28	24.09	25.46	20.43	19.27	23.41

[1] No dilution.
[2] 1:4 dilution.
[3] 1:5 dilution.
[4] 1:6 dilution.
[5] 1:10 dilution.
[6] 20 per cent solution.
[7] 12 hours=0.98 per cent; 14 hours, no change.
[8] 12 hours=25.70 per cent; 14 hours=25.88 per cent; 16 hours, no change.
[9] 12 hours=23.90 per cent; 14 hours=24.09 per cent; 16 hours, no change.

From Table 1 it may be noted that the three methods give concordant results in the hands of the individual chemist, and a majority of the cooperators have been able to check fairly well with one another. The immersion refractometer seems to give results which are much lower than the other two methods, especially so on the dark molasses. This instrument proves useful for the determination of moisture in sugars and very light colored products. Too few figures are at hand, however, to allow of any recommendation further than that more work be done.

Table 2.—*Comparative results on polarizations of molasses.*

Analyst.	Polarization of first molasses.			Polarization of blackstrap molasses.		
	Official method.	Oxalic method.	Direct true sucrose.	Official method.	Oxalic method.	Direct true sucrose.
W. E. Cross, New Orleans, La.	[1] 44.45	[1] 44.25	[1] 43.8	[2] 31.72	[1] 32.04	[1] 31.20
W. G. Taggart, New Orleans, La.	[1] 44.15	[1] 43.85	[1] 43.8	[2] 32.70	[1] 31.90	[1] 31.6
W. J. McGee, New Orleans, La.	43.45	[1] 43.70	43.2	30.60	29.9	30.5
E. H. Grant, New Orleans, La.	41.15	[1] 41.8	42.2	30.40	29.6	30.8
H. E. Z. Perkins, New Orleans, La.	44.2	[1] 44.1	44.0	33.3	32.7	28.4
Geo. P. Meade, Gramercy, La.	44.03	.....	43.1	29.99	.....	30.4
G. B. Williamson, Gramercy, La.	44.36	.....	.....	29.75	.....	.....
S. F. Sherwood, Washington, D. C.	[2] 43.42	[2] 43.50	.....	[3] 32.56	[3] 30.46	.....
G. J. Hough, Washington, D. C.	[2] 43.57	[2] 42.90	.....	[3] 28.94	[3] 30.85	.....
	43.64	43.44	43.34	31.10	31.06	31.12

[1] 26:200 dilution. [2] 26:100 dilution. [3] 26:400 dilution.

The results in Table 2 are very gratifying, especially since two of the methods are new. The average results on first molasses are as close as could be expected, and by eliminating one result under blackstrap averages are obtained which are fair for so dark a product.

The oxalic-acid method has three advantages: First, better clarification; second, the single polarization in slightly acid solution; third, no base is left in the solution to use up hydrochloric acid during inversion. A. H. Bryan said that the modified Clerget method is a tedious one, and that it is hard to tell when to stop adding oxalic acid. The writer found that after much experience with the method it was fairly easily worked.

The direct sucrose method requires less time than the Clerget method and no use of formulæ to obtain per cent of sucrose. Mr. Meade stated that the direct sucrose determination with sodium hydroxid and peroxid of hydrogen is simple and rapid, and with a little practice the effervescence would not be very troublesome.

It is recommended that both the modified Clerget and the direct sucrose method be tested further.

REPORT OF THE SEVENTH INTERNATIONAL COMMISSION FOR UNIFORM METHODS OF SUGAR ANALYSIS, HELD IN NEW YORK, SEPTEMBER 10, 1912.

By A. H. Bryan.

At the meeting of the Seventh International Commission for Uniform Methods of Sugar Analysis, held in New York on September 10, 1912, the question of standard temperature of polarization was taken up. It was unanimously voted that polarizations should be made at 20° C., as this is the temperature at which the polariscopes are standardized.

A resolution was also passed providing "that where white light is used in polarization the same shall be filtered through a neutral bichromate of potash solution of such a strength that the per cent composition multiplied by the length of column in centimeters be 9 inches."

Dr. C. A. Browne[1] has called this association's attention to the needs of this light filter cell when polarizing light-colored solutions. It is a fact that the polarizations without such a cell will be higher from 0.1 to 0.3° V. than when it is used, the correct reading being the one obtained by the use of this light filter cell. For sucrose solutions it has been found that a 3 per cent solution of bichromate in a 3 cm cell is sufficient, but for commercial glucose the percentage should be double—that is, 6 per cent in a 3 cm cell.

It seems to me that this association should adopt resolutions similar to those adopted by the International Commission for Uniform Methods of Sugar Analysis, as we find in Bulletin 107, Revised, page 39, the general directions of the international commission for polarizations and to which these two cited resolutions will be attached this year.

REPORT OF COMMITTEE B ON RECOMMENDATIONS OF REFEREES.

By E. M. Chace, *Chairman*.

(Dairy products, foods and feeding stuffs, sugar, tannin, and medicinal plants and drugs.)

Dairy Products.

It is recommended—

(1) That the subjects now under consideration be given further study.

Approved for further study.

[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 122, p. 222.

Foods and Feeding Stuffs.

It is recommended—

(1) That the method for determining acidity proposed by the previous referee (Bul. 152, p. 198) be adopted as a provisional method.

Finally adopted as provisional.

Acidity of feeds.—Weigh 10 grams of the sample into a shaking bottle, add 200 cc of distilled water, and shake for 15 minutes. Filter the extract through a folded filter and take an aliquot of 20 cc (equal to 1 gram of the sample) for the titration. Dilute with 50 cc of distilled water and titrate with standard decinormal sodium hydroxid solution, using phenolphthalein as indicator.

(2) That the acidity of feeding stuffs be reported in terms of decinormal sodium hydroxid required for neutralization, and that this method of reporting results be adopted as provisional.

Approved for final action as provisional in 1913, although recommended in 1911.

(3) That the petroleum ether method for the determination of fat, especially the comparison of the effects of petroleum ether derived from different crude petroleums, the rate of extraction, and the effect of moisture in the samples (Bul. 152, p. 198; Cir. 90, p. 8) be further studied.

Approved for further study.

(4) That in the petroleum ether method for fat the referee's recommendation that the word "distilling" be substituted for "boiling," making the method read "Extract 2 to 5 grams of the sample without previous drying for 3 hours in a Soxhlet apparatus with petroleum ether distilling below 65° C.," be not adopted.

Recommendation of committee adopted.

(5) That an associate referee be appointed to study the questions of the proper factor for the conversion of nitrogen into protein and the acidity in feeding stuffs with special reference to eliminating the error due to proteins, if these problems are of sufficient importance.

No action.

Sugar.

It is recommended—

(1) That the modified Clerget and the direct sucrose methods be tested further.

Approved for further study.

(2) That the resolutions of the International Commission for Uniform Methods of Sugar Analysis of the Eighth International Congress of Applied Chemistry concerning the temperature of polarizations and the use of a neutral potassium bichromate light filter cell be referred to the referee on sugar for a report at the next meeting.

Approved.

(3) That to the official aerometric method for the determination of total solids (Bul. 107, Rev., p. 65) a note be added to the effect that the results on molasses and other materials containing large amounts of invert sugar or nonsugar solids are only roughly approximate.

Not considered. (Available for final action in 1913.)

Medicinal Plants and Drugs.

It is recommended—

(1) That the subjects now under consideration be given further study.

Approved for further study.

REPORT OF COMMITTEE ON NOMINATIONS.

Mr. W. A. Withers, chairman of the committee on nominations, presented the following report:

The committee appointed to nominate officers for the ensuing year would respectfully report the following nominations:

President, G. S. Fraps, College Station, Tex.; vice president, E. F. Ladd, Agricultural College, N. Dak.; secretary, W. D. Bigelow, Washington, D. C.; additional members of the executive committee, C. H. Jones, Burlington, Vt.; R. N. Brackett, Clemson College, S. C.

The secretary was instructed to cast the unanimous vote of the association for the officers as nominated.

The referees and associates appointed by the outgoing executive committee were announced. They are given on pages 228 to 229 with such subsequent changes as were necessitated by declinations, etc.

Mr. B. B. Ross made a motion, which was adopted, that a committee be appointed to draft suitable resolutions with regard to the two deceased members, M. A. Scovell and H. A. Weber. The following committee was appointed: B. B. Ross, of Alabama, chairman; C. H. Jones, of Vermont; E. W. Magruder, of Virginia.

Mr. McGee made a motion that the associate refereeship on condiments other than spices be dropped and the methods under that subject be distributed among other referees by the main referee. The motion was carried by a vote of 7 to 2.

Mr. McIntire reported that the auditing committee had found the report of the secretary-treasurer correct. The report was then read.

REPORT OF THE SECRETARY-TREASURER FOR THE YEAR 1911–1912.

During the year Dr. Wiley, who filled the position of Secretary-Treasurer for over 22 years, resigned and the undersigned was appointed in his place. I have, therefore, given separately a statement of the expenditures made by Dr. Wiley since the report for 1911 and before his resignation, and a detailed statement of the receipts and expenditures made since that time.

On December 26, 1911, the date of the statement by Dr. Wiley referred to above, 22 organizations had paid their membership dues for the year. Since that date 22 additional organizations have paid their dues. A full list of the organizations whose dues have been paid is given as Exhibit C. After the statement of the treasurer, dated December 26, 1911, a bill of Byron S. Adams for circulars and envelopes amounting to $8.50 and dated December 29, 1911, was paid. The receipted bill accompanies this report, marked Exhibit D.

Respectfully submitted.

W. D. Bigelow, *Secretary-Treasurer.*

The undersigned have examined the exhibits representing the financial transactions of the association since its last annual meeting, and find them to be correct.

Burt L. Hartwell.
W. H. McIntire.
R. C. Thompson.

Mr. Veitch reported for the referee on tannin, J. S. Rogers, that there had been no cooperation with other members of the association, the work being limited to the Leather and Paper Laboratory of the Bureau of Chemistry. He stated that the present methods are very satisfactory and that he had no suggestions for improvement at that time.

Dr. H. W. Wiley, having been introduced as honorary president of the association, delivered an address, contrasting the society as it now is with its condition in 1884, when it was organized. Then there were but 10 or 12 members; to-day it has an enrollment of two or three hundred. At first it was concerned only with analytical chemistry; now it is a research as well as an analytical body, investigating great problems affecting the very foundations of agricultural science. Although the association has done more for the farmer than any other body, it must continue efficiently and patiently to work out the various problems as they arise, that agriculture may have the full benefit of science.

Mr. Barnard announced that the fortieth annual meeting of the American Public Health Association was being held that afternoon and that an invitation had been extended to all members of the Association of Official Agricultural Chemists to attend all sessions.

The appointment of the following committees was announced:

Additions to the committee on food standards: R. E. Doolittle, Washington, D. C.; B. B. Ross, Auburn, Ala.; H. E. Barnard, Indianapolis, Ind.

Committee on practicability of organizing for study of vegetable proteins: L. L. Van Slyke, Geneva, N. Y.; J. S. Chamberlain, Amherst, Mass.; J. M. Bartlett, Orono, Me.

REPORT ON MEDICINAL PLANTS AND DRUGS.

By L. F. Kebler, *Referee.*

In comparison with some of the problems under consideration by this association the subject of drugs is new, but it is gratifying to report that during the past year marked interest has been shown by collaborators throughout the United States on the subjects studied, and it is hoped that interest will not wane in the future. Cooperation was received from manufacturers, trade chemists, and State and Government officials. The interest shown has been most commendable, all appearing to feel the necessity of doing more cooperative work with a view to elaborating new methods and placing some of the present ones on a more satisfactory basis. The interest manifested has not been especially along any particular line but in all problems submitted.

Methods of Sampling.

In considering the various subjects presented during the last few years the referee has been impressed with the necessity of systematically studying the subject of sampling drugs and chemicals. The importance of procuring representative samples for analytical work is recognized by all chemists and pharmacognosists, and it appears

to be underestimated only by those who do not look with much favor upon analytical results. The skepticism at times manifested by business men is not always without reason, yet these doubters are being gradually converted or eliminated. Many are recognizing the necessity of replacing "rule of thumb" and haphazard methods by scientific procedures in the hands of competent chemists. Those who do not read the signs of the times correctly will gradually be eliminated from the race. The object of securing representative samples from each consignment, purchase, or delivery is self-evident, as without such samples the analytical results are worthless. The procuring of such samples requires large experience and good judgment on the part of the sampler. The adoption of a carefully studied and practicable procedure for taking samples would undoubtedly result in eliminating unnecessary work and at times friction and dissatisfaction. The referee would, therefore, suggest the appointment of a committee to consider the subject and report at the next annual meeting.

In 1905 the writer presented a contribution on the subject of sampling drugs and chemicals [1] in order to interest others in this fundamental subject, that they might contribute from their experience from time to time so that ultimately satisfactory procedures might be established for sampling. Since that time very little has appeared upon the subjects specifically under consideration and there is no information available in textbooks on analytical or industrial chemistry. The subject is seldom if ever considered in a college curriculum.

Many papers have been written on the subject of sampling minerals, such as ores and coal, one of the earliest contributions along this line being by A. N. Tate.[2] Among the most recent articles may be mentioned the paper by V. Samter,[3] which is accompanied by a fairly good bibliography on the subject. Mr. E. G. Bailey submitted an exhaustive contribution on the subject entitled Accuracy in Sampling Coal.[4] Notwithstanding the numerous suggestions and contributions presented on the sampling of minerals, it appears that the sampling question in conjunction with these products is far from satisfactory, as will be seen by reading a paper by Morton Webber entitled Unavoidable Errors in Sampling.[5] Mr. Bailey in his summary includes the following: "The present methods of sampling coal are almost as various as is the number of persons taking such samples, with the result that errors of 3 to 5 per cent in ash are of ordinary occurrence and extreme errors of 15 to 30 per cent are frequently encountered. * * * The chances of error in the chemical analysis are insignificant when compared with the errors in the existing methods of sampling coal. The chemist should be responsible for the sampling in every possible instance." The principles involved and discussed by the writers referred to above may be considered in conjunction with the sampling of certain drug commodities, but as a general rule are either inapplicable or must be materially modified.

Recommendations for the sampling of butter, cheese, and soils will be found in Bulletin 107, Revised, Bureau of Chemistry. Preparation of the samples previous to analysis is also given in that bulletin in connection with many of the commodities considered. General directions for sampling sugar, molasses, spirits, wines, liquors, etc., will be found in Customs Regulations of the Treasury Department for 1899 and 1908.

A method for sampling crude glycerin constitutes a part of a report of a committee on the general subject of the analysis of crude glycerin.[6] Its basis resides chiefly in a sampler devised for the purpose of replacing the glass tube formerly employed. "It consists of two brass tubes, one fitting closely inside of the other. A number of ports are cut out in each tube in such a way that when the ports are opened a continuous

[1] Proc. Amer. Pharm. Assoc., 1905, *53:* 348.
[2] J. Soc. Chem. Ind., 1884, *3:* 339.
[3] Chem. Ztg., 1908, *32:* 1209, 1224, 1250.
[4] J. Ind. and Eng. Chem., 1909, *1:* 161.
[5] Min. and Sci. Press, 1911, *102:* 846.
[6] J. Soc. Chem. Ind., 1911, *30:* 556.

slot is formed which enables a complete section to be taken throughout the entire length of the drum. By this arrangement the sampler is filled almost instantaneously with the glycerin. There are also a number of ports cut at the bottom of the sampler which render it possible to take a proportion of the salt at the bottom of the drum." This instrument could be used with advantage in sampling many of the numerous fluid drug preparations.

In 1906 H. W. Wiley, as chairman of subcommittee 4 of the International Congress of Applied Chemistry, made a report[1] covering the various subjects in general. As chairman he sent a letter to the members of his committee asking them to assist in establishing principles for sampling material for analysis. The queries addressed to each member were as follows:

(1) What in your opinion should be the extent of sampling in regard to the number of packages examined in order to secure as nearly as possible a representation of the whole?
(2) What precautions should be taken in sampling to prevent changes in the moisture content, and how should samples be secured when taken to prevent evaporation or absorption of moisture until opened for analysis?
(3) How finely divided should samples of different materials be in order to secure as nearly as possible a representation of the average quality?
(4) What particular method should be employed in securing samples of liquid products?
(5) What particular method should be employed in securing samples of viscous, elastic, or unusually tough substances which present difficulties in reducing to a homogeneous mass?
(6) Give your views in regard to the process of sampling bodies which present unusual difficulties, specifying the character of the bodies and method which you prefer in each case.
(7) Give your general views in regard to the methods of sampling which you think should be adopted by the International Committee on Analyses.

Not a single response was received to these queries, which appears to indicate general apathy on the part of chemists dealing with this subject. Why there should be this apparent indifference is difficult to comprehend. They all fully realized that if samples do not represent the total average of a given consignment the analytical results can not be relied upon as setting forth the quality of the goods under consideration.

H. E. Sindall[2] considered the subject of sampling spices, and dealt with two methods to be employed for different kinds of spices.

One of the factors contributing largely to the uncertainty of obtaining average samples is the practice of intrusting sampling to workmen or men inexperienced in the art. If such a practice is necessary the chemist should present himself from time to time as sampling is progressing for the purpose of making a careful inspection. The reason why chemists themselves can not undertake the sampling in person is an economic one, and this brings us to the subject of the number of samples to be taken from each consignment, delivery, or purchase. It is virtually impossible for economic reasons to take samples from each of 300 or 400 bales that may constitute a single consignment, or even a less number of packages, from 50 to 100. The writer's experience is that no hard and fast rule can be laid down covering all sampling of drugs, but some general procedure should be adopted which is fair and just to all parties concerned.

The minimum number of packages to be sampled on imported merchandise is clearly established by Federal law,[3] which reads as follows:

The collector shall designate on the invoice at least one package of every invoice, and one package at least of every ten packages of merchandise, and a greater number should he or either of the appraisers deem it necessary, imported into such port, to be

[1] Proc. 6th Inter. Cong. Applied Chem., 1907, 7: 169.
[2] Am. J. Pharm., 1910, 82: 80.
[3] Revised Statutes of the United States, 1878, 2d ed., p. 562, sec. 2901.

opened, examined, and appraised, and shall order the package so designated to the public stores for examination; * * *.

In practice it is often necessary to sample a larger number of packages than the minimum prescribed, and the sampling of one bale, barrel, or package in five has given very satisfactory results. In the case of high-priced commodities such as opium and other potent medicinal agents it often becomes imperative to examine every package, bale, or bag.

SAMPLING OF DRUGS.

It can be readily seen that nothing definite has been established in the case of drugs as to the number of packages to be sampled, except as to the minimum number prescribed by the law. In the case of large shipments of 100 packages or more of the ordinary crude drugs, such as leaves, roots, barks, herbs, etc., the sampling of one package in ten is satisfactory. If, however, the material should barely comply with the standard prescribed, or fall slightly below such standard, it may be necessary to examine a larger number. In such cases one package in every five should be satisfactory. In the case of commodities difficult of sampling, such as asafetida, it is desirable at the outset to sample thoroughly at least every fifth package. Thus far only the number of packages to be sampled has been considered.

The most important feature, however, is the procedure of sampling, which must be thoroughly and carefully studied for each class of goods after having decided upon the number of packages to be sampled. In the case of the consignment of leaves, for example, it will be necessary to have available specific details as to how the samples should be taken. It is admitted by all that the most satisfactory procedure is the entire opening of all the packages, so that the contents of each package would be exposed to view. Such a course, however, is in many cases impracticable, but should be resorted to whenever possible. In the case of bales of leaves or bags of seeds small portions of the material should be drawn from the upper and lower parts of the bale or bag of opposite sides as well as from the center, and small samples so drawn should be mixed, so as to produce one composite sample. It is clearly apparent that the procedure to be adopted in the sampling of drugs must be varied according to the character of the commodities. For convenience and as a basis, the following classification is suggested:

(1) Gums and gummy substances: Asafetida, benzoin, curaçao aloes, myrrh, opium, etc.
(2) Large crystals and crystalline masses: Ammonium carbonate, copper sulphate, corrosive sublimate, potassium cyanid, sodium carbonate, etc.
(3) Powders and small crystals: Acetanilid, arsenious oxid, bismuth subnitrate, bleaching powder, charcoal, cinchona bark, ipecac root, gum arabic, wild-cherry bark, etc.
(4) Crude vegetable drugs: Aconite root, belladonna leaves, bloodroot, coca leaves, cubeb berries, nux vomica beans, vanilla beans, etc.
(5) Liquids: Acetone, alcohol, ammonia water, balsam copaiba, glycerin, sulphuric acid, turpentine, etc.
(6) Articles that congeal in part or as a whole at moderately low temperatures: Oils of anise seed, eucalyptus, peppermint, sassafras, glacial acetic acid, etc.
(7) Solid at ordinary temperatures but liquefying on warming: Carbolic-acid crystals, beeswax, ceresin, Japan wax, petrolatum, paraffin, spermaceti, etc.

METHODS OF ANALYSIS.

In order to arrive at a fair conclusion relative to the purity, adulteration, or misbranding of an article, it is necessary to take into consideration all factors that may throw light upon the subject. Strictly speaking, there are comparatively few methods of analysis recognized as having a legal standing in the United States. Those now so recognized are embodied in the United States Pharmacopœia, National Formulary, and Bulletin 107, Revised, of the Bureau of Chemistry. These do not include many methods that must be called into service. The Pharmacopœial methods of analysis are usually

devised for special commodities and are often not applicable to many products met with; for example, the Pharmacopœia directs the alcohol in white wine to be determined by a certain procedure which is not applicable in many cases met with in practice. The result is that the method must be modified for each particular class of preparations. Again, the method for determining morphin in opium or laudanum is inapplicable in the case of paregoric.

In the case of chemicals and crude plant drugs the first feature that presents itself is naturally the physical appearance. If there is deviation from the normal, suspicion is aroused immediately in the mind of the trained chemist or pharmacognosist. Taste and odor have not assumed sufficient importance to warrant material recognition in analytical work, but anyone familiar with these two factors in connection with drug products has at his command a valuable asset and is fortunate indeed. By one or the other or both of these factors the chemist is frequently able to shorten his analytical work very materially. Odor or taste is often the only indication which will show the presence of certain agents with any degree of certainty.

The character and purity of a simple powdered drug may be established by microscopical examination, estimation of ash, and a determination, in certain cases, of the active principles that may be present. If, however, there is a mixture of powdered drugs, the problem becomes more difficult. Microscopically it is not difficult to determine the adulteration of belladonna root with pokeroot, but chemically it is virtually impossible. It is true the amount of alkaloidal material may be low in proportion to the adulterant present, but the manipulation may be so clever as to bring the amount of the alkaloidal material contained in the product still within the requirements of the standard.

In the case of comminuted or broken drugs it is desirable and often necessary to resort to mechanical means as the method of analysis; for example, senna siftings are frequently contaminated with foreign material of various kinds, and in order to determine the relative amounts it is necessary either to make a separation by garbling, and in this way make the determination, or an entire bale or package may be run through a suitable mill, and in this way the results rapidly and conclusively determined.

Inadequate Standards.

The Pharmacopœial standard for buchu, for example, makes no provision whatever for the presence of any stems or other incidental foreign material which is likely to find its way into the drug at the time of collection. If such a standard were put into force and effect, the amount of this drug imported into the United States would be small indeed. In practice it has been found necessary to allow the presence of a certain amount of foreign material. What has been said in connection with buchu leaves also holds for many other leaves.

Imitation balsam Peru complying with the test of the Pharmacopœia in every detail has been met with, and methods for detecting it have been devised, but it is quite possible that in the future a product may be compounded so similar to the natural product as to baffle the most skilled analyst, yet it may not be identical chemically with the natural product. What is of still greater importance is the therapeutic value of the artificial as compared with the natural product. The standard for copaiba also is decidedly inadequate, for the reason that we know so little about the ultimate composition of this commodity. In order to eliminate the uncertainties it will undoubtedly be necessary to study the article from the source of production to the time of consumption. The standards for examining oils and methods for arriving at same are inadequate, as most analysts know. In fact, there is no difficulty whatever in manipulating some of the oils so as to comply with the standards prescribed, judged from the methods detailed for examination.

Again the test prescribed by the Pharmacopœia for morphin sulphate permits the presence of a large quantity of codein and smaller amounts of the other alkaloids con-

tained in opium. In case a chemist is asked to examine a sample of morphin sulphate according to the procedure outlined in the Pharmacopœia, and it complies in every respect with the requirements, he must of necessity report it satisfactory. If this morphin sulphate containing a goodly proportion of codein is used in the manufacture of morphin sulphate tablets or other mixtures in which the morphin sulphate present constitutes an important part and the analyst discovers codein, he must of necessity infer that either the original material was contaminated with this alkaloid or the product is improperly named, or it may even be misbranded in view of the fact that the codein is not declared, a condition which might cause some considerable embarrassment.

The purpose of this paper is to show the necessity of doing an extremely large amount of analytical work.

REPORT ON HEADACHE MIXTURES.

By W. O. Emery, *Associate Referee.*

During the past year attention has been directed by the Synthetic Products Laboratory of the Bureau of Chemistry to the examination of tablets, pills, and capsuled preparations designed for the treatment of headache and other ailments. While the mixtures examined have shown great diversity in the number and proportions of potent drugs present, each preparation has involved one or more of the following agents: Caffein, acetanilid, acetphenetidin, antipyrin, monobromocamphor, sodium bromid, sodium salicylate, acetylsalicylic acid, morphin, codein, heroin, and quinin, in addition to other substances less important therapeutically as well as those commonly employed as lubricants, diluents, or vehicles. As the result of such work, on both official and control mixtures, numerous analytical procedures have been gradually developed and verified, a few of which have been presented to outside analysts for trial and criticism. Heretofore such cooperative work has had to do solely with mixtures prepared in the laboratory, in which event we were never uncertain as to the quantity and proportion of potent drugs actually present in the sample submitted for examination. In this year's work, however, it was deemed expedient to send out commercial products obtained through our inspection force from three prominent houses. The preparations, involving three combinations, were in tablet form, 20 tablets being furnished each coworker, together with the prescribed analytical procedures, as follows:

Cooperative Work of 1912.

Method of analysis of cooperative mixture no. 10, containing caffein, acetphenetidin, etc.

Determine the weight of 20 tablets, powder finely, and weigh out on a small (5.5 cm) tared filter an amount equal to the average weight of 1 tablet. Wash with successive small portions of chloroform to the amount of 40 to 50 cc, sufficient at least to insure complete extraction of all caffein and acetphenetidin in the mixture, collecting solvent in a 200 cc Erlenmeyer. Distill off chloroform by means of a small flame until only a few cubic centimeters of solvent remain in the flask, using if possible in connection with this and similar operations a small spray trap, substantially like that shown on page 239 of Bureau of Chemistry Bulletin 152. Add to the residual liquid 10 cc of dilute sulphuric acid (1 volume concentrated acid to 10 of water) and digest on a steam or vapor bath until contents of flask have evaporated to about 5 cc, then add 10 cc of water and continue digestion until the residue again amounts to about 5 cc. The quantitative separation of caffein from acetphenetidin depending as it does on a complete hydrolysis of the latter into acetic acid and phenetidin, every effort should be exerted to the end that all particles of acetphenetidin which may appear on the sides of the flask be gradually made to disappear in the acid liquid by a gentle rotation of flask during the process of digestion or by the addition of a few drops of alcohol or chloroform.

Caffein.—Cool, pour, and rinse with water into a separatory funnel, so that the final volume does not greatly exceed 20 cc. Extract three times by means of vigorous

shaking with thrice the volume of chloroform, in the present instance 60 cc. After clearing, pass through a small pledget of cotton (thrust up into the delivery tube of separator) and a small (5.5 cm) filter into a 200-cc Erlenmeyer, being careful to recover any caffein which may cling to the apex of the delivery tube and edge of filter by washing with a little fresh chloroform. The pledget of cotton is removed by means of a wire and thrown into the separator on the completion of each extraction, a fresh one being introduced into the delivery tube prior to each subsequent withdrawal of solvent. Distill the chloroform from the combined extractions until the liquid is reduced to about 10 cc, then transfer to a small tared beaker or crystallizing dish by pouring and careful rinsing with small quantities of chloroform. Allow to evaporate spontaneously or at a moderate heat on a vapor bath to apparent dryness, partially covering the dish with a watch glass toward the end of the operation in order to avoid possible loss by decrepitation. Remove dish from heat immediately on the appearance of a crystalline residue. Cool in desiccator and weigh as anhydrous caffein.

Acetphenetidin.—Wash filter used to dry chloroform in the preceding operation once with 5 cc of water, receiving latter in the separatory funnel containing the aqueous acid solution of phenetidin sulphate. Treat with successive small portions of solid sodium bicarbonate until an excess of this reagent, after complete neutralization of the sulphuric acid, persists at the bottom of mixture. Now add 60 cc of chloroform and for every 100 mg of acetphenetidin known or believed to be present, 5 drops acetic anhydrid; shake for some time vigorously, allow to clear, then pass through cotton and a dry filter, as described for caffein, into a 200-cc Erlenmeyer. Distill over 50 cc of the chloroform, make up to 60 cc with fresh solvent, and extract again. Distill over as before, this time about 60 cc, then make the third and final extraction. Distill the chloroform down to about 10 cc, transfer residue by pouring and rinsing with small quantities of solvent to a tared 50 cc crystallizing dish, evaporate on steam or vapor bath to apparent dryness, finally removing any considerable excess of acetic anhydrid by repeated additions of about 1 cc of fresh chloroform, to which has been added a drop of alcohol. The acetphenetidin will finally appear as a whitish, crystalline mass, having usually a faint acetous odor. The latter will disappear completely on standing some hours in the open, or more quickly in a vacuum desiccator over lime. The residue is weighed at intervals until it suffers no further loss.

Report results in grains per tablet as well as in parts per hundred.

COOPERATIVE RESULTS ON MIXTURE NO. 10.

The results obtained and reported for mixture No. 10 have been tabulated as follows:

Cooperative results on mixture No. 10.

Analyst.	Average weight of 1 tablet.[1]	Caffein.				Acetphenetidin.	
		Crude.		Purified.			
	Gram.	*Grains.*	*P. ct.*	*Grains.*	*P. ct.*	*Grains.*	*P. ct.*
J. M. Bartlett, Orono, Me.[2]	0.4717	0.98	13.43	.....	.....	4.77	65.53
L. A. Brown, Lexington, Ky	.4781	.93	12.58	.....	.....	4.85	65.65
M. Bye, Cincinnati, Ohio	.4807	.96	12.98	.....	.....	4.85	65.33
R. W. Clough, Seattle, Wash.[3]	.4676	.96	13.26	.....	.....	4.75	65.83
W. O. Emery, Washington, D. C	.4741	.95	12.97	.....	.....	4.88	66.60
L. S. Gilbertson, Seattle, Wash.[4]	.4656	1.00	13.88	.....	.....	4.69	65.10
C. C. Le Febvre, Washington, D. C	.4713	.96	13.15	.....	.....	4.79	65.85
C. B. Morrison, New Haven, Conn.[5]	.4790	1.00	13.40	0.93	12.53	4.90	66.28
H. L. Schulz, Detroit, Mich	.4659	.99	13.83	.....	.....	4.82	67.20
C. C. Wright, Washington, D. C	.4759	.98	13.31	.....	.....	4.87	66.19
F. Heidlberg, Philadelphia, Pa.[6]	.4713	.95	.....	.....	.....	4.76	65.23
Average	.....	.97	13.28	.....	.....	4.81	65.89
Maximum	.....	1.00	13.88	.....	.....	4.90	67.20
Minimum	.....	.93	12.58	.....	.....	4.66	65.10
Difference	.....	.07	1.30	.....	.....	.24	2.10
Composition claimed by manufacturer	.....	1.00	.....	.....	.....	5.00	.....

[1] Average weight of the single tablet in grams as found by the collaborator in the 20 submitted for analysis.
[2] Most of the analytical work done by H. W. Averill.
[3] Reported by H. M. Loomis.
[4] Reported by C. W. Johnson.
[5] Reported by J. P. Street.
[6] Reported by C. E. Vanderkleed.

METHOD OF ANALYSIS OF COOPERATIVE MIXTURE NO. 11, CONTAINING CODEIN, ACETANILID, AND SODIUM SALICYLATE.

The separation and estimation of these three substances is effected in the following manner: Reduce the 20 tablets submitted to a fine powder, then weigh out an amount equal to the average weight of one tablet, transfer to a separatory funnel, add 50 cc of chloroform, 10 cc of water, and about 1 cc of dilute sulphuric acid, sufficient to insure acidity in the solution. Shake vigorously, allow to clear, then draw off solvent through a pledget of cotton and dry filter into a second separatory funnel of about 200 to 250 cc capacity. Extract a second and third time, using the same amount of chloroform as before. After each withdrawal of solvent throw the cotton pledget into the separator and substitute a fresh one in its place. Treat the combined chloroformic extracts as directed under acetanilid.

Codein.—Wash the filter used to dry the chloroform solution of acetanilid and salicylic acid with 5 cc of water, receiving latter in the separator containing the aqueous acid solution of codein. Add solid sodium bicarbonate in slight excess, then extract by means of vigorous shaking with three 50 cc portions of chloroform. Dry the solvent with cotton and filter as above, collect in a 200 cc erlenmeyer, then distill the liquid by the aid of gentle heat down to about 10 cc. Transfer the residue, by pouring and rinsing with fresh chloroform, to a small tared beaker, evaporate to apparent dryness on the steam bath, cool, and weigh as anhydrous codein. Verify by moistening the residue with a little neutral alcohol and titrating with fiftieth-normal sulphuric acid, using Methyl Red (1 drop alcohol soluble) as indicator.

Acetanilid.—To the chloroform solution of acetanilid and salicylic acid add 20 cc of water and, for every 100 mg of salicylic acid known or believed to be present, 1 gram of anhydrous sodium carbonate. Shake vigorously, allow to clear, withdraw the chloroform through cotton, and filter into a 200 cc erlenmeyer. Extract the aqueous-alkaline solution with two 10 cc portions of chloroform in order to remove traces of acetanilid taken up temporarily by the water. Distill the united chloroformic extracts by the aid of gentle heat down to about 10 cc. Introduce into flask 10 cc of dilute sulphuric acid (1:10 by volume) and digest on the steam bath until the residue amounts to 3 to 5 cc, then add 20 cc of water and 10 cc of concentrated hydrochloric acid. Into this solution run slowly a standard solution of potassium bromid-bromate until a distinct yellow coloration persists. Multiply the number of cubic centimeters required to complete the formation of tribromanilin by the value of 1 cc in terms of acetanilid to ascertain the amount of this substance originally present in the sample taken.

Sodium salicylate.—Transfer the aqueous soda solution containing the salicylic acid from the separator to a 200 cc erlenmeyer, dilute with water to about 100 cc, heat nearly to boiling, then run in from a burette 25 to 50 cc of fifth-normal iodin in potassium iodid (or double this quantity of tenth-normal strength) sufficient to insure an excess of this reagent. Digest the product on the steam bath for one hour, adding iodin solution from time to time in order to maintain a slight excess. The reddish, insoluble precipitate has the composition $(C_6H_2I_2O)_2$, being identical with the "red substance" first described by Lautemann [1] and later used by Bougault [2] in the separation of salicylic from benzoic and cinnamic acids. Remove any excess of iodin by the addition of a few drops of sodium thiosulphate solution, decant liquid through a tared gooch, care being taken that most of the precipitate remains in the flask. Add 50 cc of boiling water to the latter, digest 10 minutes on steam bath, then pour into gooch, into which all the reddish precipitate is gradually washed, using for this purpose and subsequent washings about 200 cc of hot water. Dry in air bath to constant weight. The weight of precipitate multiplied by 0.4658 will give the amount of sodium salicylate originally present in the sample.

Should acetphenetidin instead of acetanilid be employed in the above combination, the procedure need be modified only to this extent, that the chloroform solution of acetphenetidin and salicylic acid, after elimination of the latter substance through treatment with sodium carbonate, is distilled down to about 10 cc and the residue transferred to a small crystallizing dish for final evaporation of solvent and weight of resulting acetphenetidin.

In this connection it may not be amiss to state that a quantitative separation of codein, acetanilid, and sodium salicylate could be effected in substantially the

[1] Liebig's Annalen, 1861, *120:* 309.
[2] J. Pharm. Chim., 1908, *28:* 145.

following manner: To the mixture in separatory funnel add about 100 mg of sodium bicarbonate, 10 cc of water, and 50 cc of chloroform. Extract three times with this quantity of solvent. The sodium salicylate remaining in separator is transferred to an erlenmeyer and treated with iodin solution as above directed, after the addition of the requisite amount of solid sodium carbonate (anhydrous). The separation of codein and acetanilid is accomplished by shaking out the chloroform solution of these two substances with 10 cc of dilute sulphuric acid. The acid solution is washed twice thoroughly with about 10 cc of chloroform in order to remove traces of acetanilid taken up temporarily by the aqueous medium. The latter substance, after removal of solvent and subsequent hydrolysis, is titrated with standard bromid-bromate solution, as already described. The aqueous-acid solution of codein is treated with solid sodium bicarbonate in excess, the alkaloid thereupon extracted with chloroform. On removal of solvent by distillation and evaporation the codein is first weighed and then titrated with fiftieth-normal sulphuric acid.

COOPERATIVE RESULTS ON MIXTURE NO. 11.

The results obtained and reported for mixture No. 11 have been tabulated as follows:

Cooperative results on mixture No. 11.

Analyst.	Average weight of 1 tablet.[1]	Codein.				Acetanilid.		Sodium salicylate.	
		Gravimetric.		Volumetric.					
	Gram.	*Grain.*	*Per ct.*	*Grain.*	*Per ct.*	*Grains.*	*Per ct.*	*Grains.*	*Per ct.*
J. M. Bartlett, Orono, Me.	0.4969	0.27	3.58	.....	.....	2.55	33.27	1.90	24.85
L. A. Brown, Lexington, Ky.	.4953	.24	3.18	0.23	2.97	2.88	37.61	1.89	24.72
R. W. Clough, Seattle, Wash.[2]	.5000	.21	2.67	.....	.....	2.90	37.52	1.95	25.25
W. O. Emery, Washington, D. C.	.4969	.24	3.24	.....	.....	2.91	38.04	1.89	24.71
L. S. Gilbertson, Seattle, Wash.[3]	.4925	.25	3.32	.24	.....	2.87	37.76	1.89	23.55
F. Heidlberg, Philadelphia, Pa.[4]	.4977	.25	3.28	.....	.....	2.84	36.93	1.92	25.02
C. C. Le Febvre, Washington, D. C.	.4969	.....	.....	.25	3.31	2.90	37.83	1.95	25.46
C. B. Morrison, New Haven, Conn.[5]	.4970	.25	3.21	.....	.....	2.95	38.45	1.96	25.73
C. D. Wright, Washington, D. C.	.5023	.24	3.11	.22	2.84	2.86	36.83	1.97	25.37
Average	.....	.25	3.20	.....	.....	2.85	37.14	1.92	24.93
Maximum	.....	.27	3.58	.....	.....	2.96	38.45	1.97	25.73
Minimum	.....	.21	2.67	.....	.....	2.53	33.27	1.87	23.55
Difference	.....	.06	.91	.....	.....	.43	5.18	.10	2.18
Composition claimed by manufacturer	.....	.25	.....	.....	.....	3.00	.....	2.00	.....

[1] Average weight of the single tablet in grams as found by the collaborator in the 20 submitted for analysis.
[2] Reported by H. M. Loomis.
[3] Reported by C. W. Johnson.
[4] Reported by C. E. Vanderkleed.
[5] Reported by J. P. Street.

METHOD OF ANALYSIS OF COOPERATIVE MIXTURE NO. 12, CONTAINING ANTIPYRIN, ACETPHENETIDIN, AND CODEIN SULPHATE.

The problem presented in this combination, in so far as the separation of the several ingredients is concerned, is very similar to that obtaining in a mixture of caffein, acetphenetidin, and codein sulphate. Antipyrin behaves toward chloroform very much like caffein, in that it can with equal facility be recovered from alkaline, neutral, and acid solutions. Owing to its very high solubility, however, in aqueous media, a complete extraction therefrom can only be accomplished by a greater number of shake outs than is required for caffein. Antipyrin may be estimated gravimetrically as in the case of caffein, or volumetrically by means of a standard alcoholic solution of iodin in the presence of mercuric chlorid. Its separation from acetphenetidin or acetanilid is easily effected by means of chloroform, after subjecting a mixture to hot digestion with dilute sulphuric acid, whereby the arylamids are changed into the corresponding sulphates.

Ascertain the weight of the 20 tablets submitted, then weigh out an amount of the finely powdered sample equal to the average weight of one tablet, transfer to a separatory funnel, add 50 cc of chloroform, 10 cc of water, and a few drops of dilute sulphuric acid. Shake vigorously, allow to clear, then draw off through a pledget of cotton and a small dry filter into a 200 cc erlenmeyer. Repeat the extraction four times, distilling off a portion of the solvent on completion of the third shake out, in order to accommodate the chloroform from the final two extractions in the same flask. On completion of the fifth and final shake out, distill the chloroform down to about 10 cc, add 10 cc of dilute sulphuric acid (1:10 by volume), continue heating gently until all the solvent has passed over, remove to steam bath, and digest till the volume of liquid amounts to about 5 cc, add 10 cc of water, and continue the treatment until the liquid is again reduced to 5 cc. In order that the hydrolysis may be complete; that is, no particles of acetphenetidin remain on the sides of the flask, rotate the latter gently from time to time during the process of digestion, or, better perhaps, add a few drops of alcohol or chloroform now and then.

Antipyrin.—Transfer the aqueous acid solution of phenetidin sulphate and unchanged antipyrin to a separatory funnel by the aid of water, so that the final volume does not exceed 20 cc. Make seven extractions with 30 cc portions of chloroform, clearing and drying the solvent by means of a cotton pledget, and dry filter as hereinbefore described. Distill the united chloroformic extractions down to about 10 cc, transfer to a tared 50 cc beaker, evaporate on a steam bath to apparent dryness; cool, and weigh. It has been found that antipyrin retains traces of moisture and chloroform with great obstinacy, hence several days are required, even with the help of a vacuum desiccator, to approximate to a constant weight. It is therefore more expeditious to subject the crude residue to volumetric estimation. To this end dissolve the antipyrin in 95 per cent alcohol, transfer to a 100 cc graduated flask, and fill to the mark. Titrate an aliquot (20 cc or more if the amount of antipyrin is relatively small) of the alcoholic solution of antipyrin thus prepared with an iodin solution prepared and standardized in the following manner: Make up three solutions—A, containing 1.351 grams of resublimed iodin in 100 cc of 95 per cent alcohol; B, containing 1 gram of pure antipyrin in 100 cc of 95 per cent alcohol; C, containing 5 grams of mercuric chlorid in 100 cc of 95 per cent alcohol. Into a 100 cc erlenmeyer measure from a burette 20 cc of Solution B, and by means of a pipette 10 cc of Solution C, then run in from a burette the iodin solution until a faint yellow coloration persists after a lapse of 3 to 5 minutes. Run several duplicates and it will be found that, under the conditions obtaining in the experiment, approximately 20 cc of the iodin solution will be required to bring about this result; in other words, 1 cc of iodin solution thus standardized will very nearly correspond to 1 cc of antipyrin solution, or, by weight, 10 mg of antipyrin. The value of the iodin solution in terms of antipyrin having been thus determined, subject the aliquot of the alcoholic solution of antipyrin from sample under examination to a similar treatment, basing the final result on an average of two or more titrations.

Since the presence of either acetphenetidin or codein is apparently no bar to the accuracy of the above reaction, it is wise to check the value for antipyrin already obtained by treating the original sample as follows: Weigh out on a small (5.5 cm) filter an amount of the powdered sample equal to the average weight of one tablet, wash with successive small portions of 95 per cent alcohol, in quantity about 20 to 30 cc, sufficient at least to remove all the antipyrin in the mixture. Collect solvent

in a 100 cc erlenmeyer, add 10 cc of Solution C, then run in the standard iodin until a faint yellow coloration persists. The number of cubic centimeters of iodin required to effect this should agree approximately with the value previously obtained.

Acetphenetidin.—Wash the filter used to dry the chloroform solution of antipyrin once with 5 cc of water, allowing the latter to run into the separator containing the phenetidin sulphate. Treat the solution with successive small portions of solid sodium bicarbonate until an excess of this reagent, after complete neutralization of the sulphuric acid, persists at bottom of the liquid. Now add 60 cc of chloroform and, for every 100 mg of acetphenetidin known or believed to be present, about 5 drops acetic anhydrid; shake for some time vigorously, allow solvent to clear, then pass through cotton, and dry filter into a 200 cc erlenmeyer, exactly as in the extraction of caffein. Distill over 50 cc of the chloroform, make up to 60 cc with fresh solvent, and extract again. Draw off chloroform, distill as before, this time about 60 cc, then make a third and final extraction. Distill to about 10 cc, transfer by pouring and rinsing with small quantities of chloroform to a tared 50 cc crystallizing dish, evaporate on the steam bath to apparent dryness, finally removing any considerable excess of acetic anhydrid by repeated additions of about 1 cc of fresh chloroform, containing 1 to 2 drops alcohol. The acetphenetidin should finally appear as a whitish, crystalline mass, having usually a faint acetous odor. This will entirely disappear on standing some time in the open, or more quickly in a vacuum desiccator over lime. The residue should be repeatedly weighed until it suffers no further loss.

Codein sulphate.—The estimation of codein can be carried out during the hydrolysis of acetphenetidin. Wash the filter used to dry the chloroform solution of antipyrin and acetphenetidin once with 5 cc of water, receiving latter in the separator containing the aqueous-acid solution of codein. Add solid sodium bicarbonate in slight excess, then extract three times with 50 cc of chloroform. Clear, dry, and collect solvent from the three operations in a 200 cc erlenmeyer, then distill down to about 10 cc. Transfer to a 50 cc tared beaker, evaporate to apparent dryness on the steam bath, moisten the amorphous residue with a few drops of alcohol, evaporate again, cool, and weigh. This weight, multiplied by the factor 1.3144, will give the amount of codein sulphate originally present in the sample. It is recommended that this result, which is likely to be somewhat high, be verified by titrating the crude codein with fiftieth-normal sulphuric acid, using if available 1 drop of methyl red solution as indicator.

Comments and suggestions.—In the event that acetanilid instead of acetphenetidin is employed in combination with antipyrin and codein sulphate, it would be necessary to modify the procedure only as regards the final treatment of the aqueous acid solution containing in addition to antipyrin the acetanilid, which after being subjected to hydrolysis and freed from the former is titrated with potassium bromid-bromate.

Manseau [1] in studying the action of aqueous iodin on antipyrin found that 1 gram of the latter substance absorbs at a temperature of about 40° 0.08636 iodin. He proposed to utilize this fact as a basis for a proximate assay of antipyrin. Bougault [2] suggested titration with alcoholic iodin in the presence of mercuric chlorid. He found after considerable experimentation that one molecule of antipyrin absorbs approximately one molecule of iodin. Fernau [3] was unable to obtain satisfactory results in following Bougault's suggestions. The Hoechst Chemical Works has nevertheless used his procedure in estimating antipyrin in the presence of caffein and citric acid, and Zernik [4] has done practically the same thing in the analysis of migraenin for antipyrin. Numerous experiments made in the bureau have demonstrated that Bougault's method is dependable, hence its adoption as the basis for the procedure as above outlined. It has been found that the presence of caffein, codein, acetanilid, or acetphenetidin does not affect in any material degree the accuracy of the titration of antipyrin with alcoholic iodin.

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[1] Bull. Soc. Pharm., Bordeaux, 1889, p. 148.
[2] J. de Pharm. et Chimie, 1898 (6) 7, 161–3.
[3] Z. Allg. Oester. Apoth. Ver. 1904, No. 2.
[4] Apoth. Ztg., 1906, *21:* 686.

COOPERATIVE RESULTS ON MIXTURE NO. 12.

The results obtained and reported for mixture No. 12 have been tabulated as follows:

Cooperative results on mixture No. 12.

Analyst.	Average weight of 1 tablet.[1]	Codein sulphate.				Antipyrin.				Acetphenetidin.	
		Gravimetric.		Volumetric.		Indirect.[2]		Direct.[2]			
	Gram.	*Grn.*	*Pct.*	*Grns.*	*P. ct.*	*Grns.*	*P. ct.*	*Grns.*	*P. ct.*	*Grns.*	*P. ct.*
J. M. Bartlett, Orono, Me.	0.5519	0.28	3.20	.....	.....	4.45	52.29	.....	.....	2.04	23.95
L. A. Brown, Lexington, Ky.	.5537	.30	3.53	.....	.....	4.76	55.69	4.85	56.73	2.02	23.59
R. W. Clough, Seattle, Wash.[3]	.5509	.22	2.62	.....	.....	4.57	53.85	.....	.....	2.07	24.32
W. O. Emery, Washington, D. C.	.5504	.24	2.81	0.24	.....	4.83	56.90	4.75	55.88	2.02	23.80
L. S. Gilbertson, Seattle, Wash.[4]	.5521	.25	2.96	.....	.....	4.74	55.57	.....	.....	1.99	23.48
F. Heidlberg, Philadelphia, Pa.[5]	.5525	.25	2.92	.....	.....	4.71	55.17	4.73	55.49	1.98	23.18
C. C. Le Febvre, Washington, D. C.	.5504	.....	.....	.23	2.59	4.66	54.74	.....	.....	2.02	23.76
C. B. Morrison, New Haven, Conn.[6]	.5510	.27	3.30	.....	.....	4.81	56.54	.....	.....	2.00	23.43
C. D. Wright, Washington, D. C.	.5504	.25	2.97	.22	2.60	4.68	55.10	4.75	55.90	2.05	24.10
Average	.....	.26	3.04	.....	.....	4.70	55.09	.....	.....	2.02	23.73
Maximum	.....	.31	3.68	.....	.....	4.83	56.90	.....	.....	2.08	24.50
Minimum	.....	.22	2.62	.....	.....	4.45	52.29	.....	.....	1.96	22.97
Difference	.....	.09	1.06	.....	.....	.38	4.61	.....	.....	.12	1.53
Composition claimed by manufacturer	.....	.25	.....	.....	.....	5.00	.....	.....	.....	2.00	.....

[1] Average weight of single tablet in sample lot examined.
[2] Antipyrin estimated after separation from eodein and acetphenetidin as well as directly in the presence of these drugs after extraction of the tablet mass with alcohol.
[3] Reported by H. M. Loomis.
[4] Reported by C. W. Johnson.
[5] Reported by C. E. Vanderkleed.
[6] Reported by J. P. Street.

DISCUSSION OF RESULTS.

The first mixture sent out, No. 10, contained as active ingredients caffein and phenacetin (acetphenetidin), the separation and estimation of which latter substance has proved a stumblingblock to not a few of our former coworkers unfamiliar with the peculiar behavior of phenacetin toward certain reagents. While the ready solubility of phenacetin in chloroform is well known to chemists engaged in pharmaceutical investigations, its behavior toward sulphuric acid is doubtless less familiar. As in the separation of caffein and acetanilid the latter must be hydrolyzed by means of dilute sulphuric acid into acetic acid and anilin (or anilin sulphate), so likewise with caffein and phenacetin, the latter must first be resolved into acetic acid and phenetidin sulphate. On the quantitative formation of this last-named product depends any exact separation of caffein and phenacetin (acetphenetidin), as outlined in the foregoing method. The desired result is possible only when sulphuric acid of certain definite concentrations is employed.

In general acylanilids, on treatment with concentrated sulphuric acid, are resolved quantitatively into the corresponding base and acid, in fact such hydrolysis is more readily effected with concentrated acid than with an alkali. Acetanilid, however, forms a notable exception to this rule. In the cold it dissolves without change in concentrated sulphuric acid.[1] Heated for half an hour at about the temperature of

[1] Ber. d. chem. Ges., 1884, *17:* 262.

boiling water, it remains for the most part unchanged. After heating 4 to 5 hours most of the acetanilid will be found in the form of sulphanilic acid, while a much smaller portion is changed into acetsulphanilic acid.[1] Of anilin not a trace remains. On heating phenacetin 1 to 2 hours with concentrated sulphuric acid, phenacetin sulphonic acid will be found as the resultant product.

In case the acid contains some water, the reaction takes an unexpected and entirely different course; the phenacetin is resolved into ethyl acetate and paramidophenol, the latter being subsequently changed into the corresponding sulphonate. It may not be amiss to state, in passing, that this property of phenacetin to yield ethyl acetate under the conditions named may be used to differentiate it from acetanilid. When phenacetin is treated with 50 per cent sulphuric acid, it dissolves with ease, the resultant clear solution, however, soon solidifying to a white crystalline mass, consisting of a sulphate which on contact with water is immediately resolved into its constituents. When, finally, phenacetin (and this touches us more nearly) is boiled or heated for a time with 50 per cent sulphuric acid or one much more dilute, it is resolved into acetic acid and phenetidin, which in form of its sulphate is insoluble in chloroform and hence readily separable from any accompanying caffein. This then is the direction any successful separation of caffein from phenacetin (acetphenetidin) must follow, if effected according to our method. A sulphuric acid, prepared by diluting 1 part concentrated acid with 10 parts water, is now recommended as being the safest concentration for the average worker, care being nevertheless taken that the evaporation be not driven too far and that water be added once or twice during hydrolysis.

The cooperative results obtained and reported on all three mixtures are very satisfactory, the deviation from the apparent true values being no more than would be expected, in view of the difficulties naturally inherent in the methods, but particularly owing to the fact that fully one-half the workers had little or no previous experience in drug analysis of this character.

Mr. Heidlberg found the hydrolysis of acetphenetidin greatly facilitated by the addition of small quantities of chloroform several times in order to dissolve the particles of that substance which are inclined to separate and cling to the sides of the flask. Mr. Vanderkleed and Mr. Loomis both call attention to the apparent necessity of using a larger quantity of chloroform in extracting acetphenetidin from the powdered tablet. Naturally extractions of this character should be continued until a drop of filtered solvent fails to leave any apparent residue when evaporated on a watch glass. Judging from the failure of the coworkers to suggest improvements on or make criticisms of methods covering mixtures Nos. 11 and 12, it is assumed that no serious difficulties were encountered in the work. Mr. Heidlberg found the process of drying the antipyrin after extraction with chloroform somewhat tedious, however, many days being required to bring the residues to constant weight. If pressed for time, as is frequently the case in commercial laboratories, one would better pass directly to the titration with iodin, instead of attempting a proximate determination of the antipyrin, that is, by weight.

Estimation of Caffein, Acetanilid, Quinin, and Morphin.

In connection with our investigation of tablets and pills, the problem was frequently presented to separate and estimate the above-named drugs. The procedure as developed on controls and finally adopted is substantially as follows:

Weigh out an amount of the powdered sample containing at least one-fifth grain (about 12 mg) of morphin, transfer to a separatory funnel, adding 20 cc of water, 10 drops of dilute sulphuric acid, and 60 cc of alcohol-free chloroform. Shake vigorously, allow to clear, then draw off through a pledget of cotton and small dry filter into a second separator, in which the solvent is washed with 5 cc of water to remove any

[1] Liebig's Annalen, 1899, *309:* 233.

alkaloidal traces that may have been taken up by the chloroform. The latter is finally passed through a small filter into a 200 cc Erlenmeyer for subsequent distillation and treatment in the separation of caffein and acetanilid.

Quinin.—Repeat the extraction of the aqueous-acid mixture with 50, 40, and 30 cc portions of chloroform, each portion of solvent being treated as directed for the first. The wash water is returned to the first separator, the liquid rendered alkaline by the addition of strong caustic soda in slight excess. We now have the morphin as sodium morphinate insoluble in chloroform, while the quinin is precipitated as a white flocculent mass. Shake out four times with 60, 50, 40, and 30 cc portions of alcohol-free chloroform, the solvent from each operation being passed through cotton and a dry filter, prior to reception in the erlenmeyer and distillation from the quinin dissolved therein. Transfer to a small tared beaker by pouring and rinsing with a small quantity of chloroform, evaporate to apparent dryness on the steam bath, heat amorphous residue one hour at 125° C. in an air bath, cool, and weigh. Dissolve in alcohol, transfer to a graduated 100 cc flask, fill to mark with water and sufficient alcohol to prevent precipitation of alkaloid; then remove with a pipette an aliquot containing at least one-tenth of the total amount of quinin present and titrate with fiftieth-normal sulphuric acid, using as indicator 1 to 2 drops of Methyl Red (100 mg dissolved in 100 cc of alcohol). The number of cubic centimeters required multiplied by 8.66 will give the number of milligrams of quinin sulphate in the aliquot titrated.

Morphin.—In order to recover the morphin from its combination with sodium, add an amount of ammonium chlorid slightly in excess of that required to react with the caustic soda previously introduced into the separator and as much common salt as the liquid will dissolve. A slight excess of the latter substance above that necessary to saturate the mixture can do no particular harm other than render the subsequent removal of the chloroform less quantitative. Extract four times by means of vigorous shaking with 45, 50, 40, and 30 cc portions of Pharmacopœial chloroform, to the first portion of which are added 5 cc of alcohol prior to extraction. Wash each portion of solvent in a second separator with about 5 cc of water, after extraction but before passing through cotton, and filter into a 200 cc Erlenmeyer for distillation. Distill off most of the solvent, transfer residue by pouring and rinsing with fresh chloroform to a tared crystallizing dish, evaporate to apparent dryness on the steam bath, treat residue with a few drops dilute alcohol should it seem desirable to obtain the morphin in crystalline form, heat again to dryness, cool, and weigh. Verify by dissolving the alkaloid in a few cubic centimeters of warm alcohol and titrating with fiftieth-normal sulphuric acid, using as indicator a drop of Methyl Red solution. The number of cubic centimeters required multiplied by 7.53 will give the number of milligrams of morphin sulphate present in the original sample.

Caffein.—Distill the chloroformic solution of caffein and acetanilid down to about 10 cc, add 10 cc of dilute sulphuric acid (1 : 10), digest on steam bath until contents of flask have evaporated to about 5 cc, add 10 cc of water, and evaporate a second time; transfer residue to a separator by pouring and rinsing with water so that the final volume does not exceed 20 cc; then make three extractions with 50 cc portions of chloroform. After clearing, pass solvent through cotton and dry filter into a 200 cc erlenmeyer; distill from the combined extractions down to about 10 cc, then transfer to a small tared crystallizing dish by pouring and careful rinsing with small quantities of fresh chloroform. Allow to evaporate spontaneously, or at a moderate heat, on a vapor bath to apparent dryness. Remove from heat immediately on the appearance of a crystalline residue. Cool in desiccator and weigh as anhydrous caffein.

Acetanilid.—Draw off the acid solution remaining in separator into the same erlenmeyer previously used in effecting the hydrolysis, wash out separator several times with water to insure complete removal of former contents; heat the aqueous-acid solution a short time on steam bath to expel all traces of chloroform; wash the filter used in the preceding operation to dry the chloroform solution of caffein once with 5 cc of water, receiving latter in the main solution of anilin sulphate; add 10 cc of concentrated hydrochloric acid, and titrate with a standard potassium bromid-bromate, 1 cc of which is equivalent to 10 mg of pure acetanilid. The number of cubic centimeters required to complete the precipitation, multiplied by the value of 1 cc in terms of acetanilid, will give the quantity of this substance originally present in the sample taken. In the event that the quantity of acetanilid present in the tablet is relatively large, as is frequently the case when compared with the morphin content, it is best to titrate only an aliquot of the anilin sulphate solution.

THE MELTING TEMPERATURE OF ASPIRIN AND SALICYLIC ACID MIXTURES.

By W. O. Emery and C. D. Wright.

Investigations carried on independently by Etescher,[1] Schweitzer,[2] Beringer,[3] and Kebler [4] have shown how the melting point of phenacetin (acetphenetidin) is influenced by the presence of other substances, notably acetanilid, a relatively small quantity of the latter being sufficient to depress the point of complete fusion markedly.

Among the various problems presented to the Synthetic Products Laboratory was the estimation of acetylsalicylic acid in "aspirin" tablets, capsules, and pills. Of the 21 different samples examined, several had a decided acetous odor on opening the original containers, an indication of partial resolution into acetic and salicylic acids. For purposes of analysis the samples were finely powdered and then exhausted with chloroform in the cold, the solvent being subsequently rapidly evaporated by means of an air blast, the crystalline residues dried 24 hours in a desiccator over lime, and weighed. The several residues were found to melt at temperatures ranging from 120° to 130° C. From the fact that various bulk samples of "aspirin" Bayer melted quite sharply at 130.5–131°, it was apparent that the depression in the melting temperature of certain samples had its rise in the presence of salicylic acid, as also of traces of hydrocarbons employed on occasion as lubricants in the process of tableting.

In the hope that a study of the melting temperatures yielded by mixtures of "aspirin" and salicylic acid in varying proportions might develop data from which conclusions could be drawn relative to the proportion of the two substances in admixture, the following experiments were carried out:

The materials employed were "aspirin" Bayer melting at 130.5° to 131°, and salicylic acid Merck melting at 158°, the examination of other brands of acetylsalicylic acid already in hand being deferred to a later date. Mixtures of these two substances in varying proportions were prepared by weighing out separate portions of each substance, transferring to an agate mortar, and grinding thoroughly, the total weight of each mixture being about 0.5 gram. The samples thus prepared were allowed to stand at least one day in a desiccator over lime, and the melting temperatures determined in capillary tubes, using a bath consisting of a Kjeldahl flask and inner test tube, both filled with sulphuric acid. The thermometer used was of the Anschuetz type, but the temperatures given are uncorrected. The rate of heating was such as to give a rise in temperature of about 1° per minute. An accurate determination of the melting temperature in this way is rendered difficult by the fact that "aspirin" decomposes on heating, as evidenced in the depression of the melting temperature of the pure substance of about 1° for every 5 minutes heating just below its melting temperature. As a check on the method of mixing by grinding and also to approximate more nearly the conditions of an actual determination (that is, by extraction from tablets), portions of about 0.2 gram of "aspirin" and of each of the 9 mixtures involved in Series I were dissolved in a little chloroform in a small beaker and the solvent then rapidly evaporated with an air blast. The residues after standing overnight in a vacuum desiccator over lime were rubbed up and the melting temperatures determined, these, given in Series II, agreeing fairly well with those obtained with the original mixtures, but being somewhat higher in general, as will appear from the table.

A third series of mixtures was prepared by weighing portions of "aspirin" and salicylic acid in small beakers, dissolving in chloroform, and then blasting off solvent as before. After being rubbed up to a fine powder and standing several days over lime, the melting temperatures were determined with the following results, duplicates being run with each sample:

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[1] Apoth. Ztg., 1888, *3:* 483.
[2] J. Soc. Chem. Ind., 1895, *14:* 852.
[3] Drug. Circ., 1903, 47, 184.
[4] U. S. Dept. Agr., Bureau of Chemistry Bul. 80, p. 39.

Melting temperatures of "aspirin" and salicylic acid samples.

Series I, dry mixed.			Series II, from chloroform solution.			Series III.			
"Aspirin."	Softens at—	Melts at—	Softens at—	Melts at—	Salicylic acid.	"Aspirin."	Softens at—	Melts at—	Salicylic acid.
Per ct.	°C.	°C.	°C.	°C.	*Per ct.*	*Per ct.*	°C.	°C.	*Per ct.*
100.0	129.5	129.5–130.5	129	129.5–130	0.0	100.0	129	129.5–130	0.0
87.4	125	127 –130	128	129 –129.5	12.6	96.4	127	128 –129	3.6
84.0	117	124 –128	123	124.5–127	16.0	90.5	127	128.5–129.5	9.5
71.6	115	117 –124	115	119 –124	28.4	76.1	116.5	120.5–123	23.9
68.6	115	118 –122	115.5	119 –123	31.4	57.2	114.5	115.6–118	42.8
57.7	115	115 –116	114.5	115 –115.5	42.3	49.9	114	115.6–127.5	50.1
49.9	114.5	116 –126	114	116 –126	50.1	35.5	116	122 –135.5	64.5
26.6	118	130 –141	122	132 –142	73.4	10.8	[1] 130	147 –152.5	89.2
17.3	[1] 120	141 –151	[1] 121	142 –151	82.7	0.0	158	158	100.0
8.4	137	147.5–152.5	141	149 –153	91.6	.....	.....	.....	.....
0.0	158	158	158	158	100.0	.....	.....	.....	.....

[1] Very slightly.

The behavior of the mixtures on heating is interesting, there being a decided tendency to soften several degrees below the melting temperature—in some cases 10° to 20°—the material then remaining in a more or less pasty condition until the point of complete fusion is reached, which in the case of mixtures containing 60 to 85 per cent of salicylic acid extends over about 10°, the acid not dissolving readily in the melt, but remaining in solid form at the bottom and sides of capillary. The sharp minimum melting point (115° to 116°) given by the mixture containing 58 per cent of "aspirin" is of interest, as indicating a eutectic mixture having about that composition.

As a means of estimating the proportion of salicylic acid in such mixtures, the determination of the temperatures of softening and fusion does not appear to be sufficiently delicate. More helpful results are hoped for in a study of the acidity values in the original tablet as well as in the crystalline residue obtained on extraction of the powdered sample with chloroform.

PRELIMINARY REPORT ON THE VOLUMETRIC DETERMINATION OF SALOL BY MEANS OF STANDARD BROMIN SOLUTIONS.

By C. C. LeFebvre.

The estimation of salol or phenylsalicylate in tablets, such as the plain salol tablets on the market, containing no other active ingredient, may be carried out by extracting the powdered sample on a small filter with chloroform, allowing the solvent to evaporate spontaneously in a tared beaker or crystallizing dish, and weighing the residue. This method is tedious, because the chloroform passes off very slowly, and it gives somewhat lower than the theoretical results, owing to the volatility of the salol. For this reason the possibility of titrating the salol, or rather its regenerated constituents, was examined by means of a standard bromin solution similar to that of the U. S. Pharmacopœia. Phenylsalicylate itself does not react readily with bromin, but since it may easily be saponified and the resulting phenol and salicylic acid are readily acted upon by halogens, the following procedure was tried:

First extract the salol from a weighed portion of the powdered sample by means of 50 cc ether. In order to prevent moisture from collecting on the paper, use a short extraction thimble in an ordinary extraction tube, the ether being introduced by means of a separatory funnel, the stem of which passes through a stopper in the end of the tube. Run the solvent into the flask provided with a reflux tube ground to fit; add 10 cc of normal sodium or potassium hydroxid. Place the mixture on a steam bath under the reflux, which allows the ether to escape very rapidly. The minimum

time necessary for complete saponification has not yet been determined, but in our experiments one-half to three-quarters of an hour was allowed. After this is complete dilute the alkaline liquid to about 200 cc, add an excess of potassium bromid-bromate solution, followed by 10 cc of concentrated hydrochloric acid. Shake the mixture for a minute, and then frequently during a half hour. At the end of this time add 10 cc of a 15 per cent potassium-iodid solution, and frequently agitate the mixture while it reacts for 15 minutes. Titrate the free iodin with thiosulphate solution which has been standardized against the bromin solution. From the number of cubic centimeters of the bromin solution expended, calculate the salol on the basis of 12 atoms of bromin to 1 molecule of salol. The bromin solution may very easily be standardized with acetanilid, a method we have employed.

In five determinations carried out as above outlined, where 0.3 gram of salol was used as the sample, the salol found varied from 99.9 to 100.2 per cent of that taken. This shows that the method is accurate. A few determinations made, where various excipients were added to the salol before saponification and allowed to remain throughout the titration, indicate that none of them except lactose interferes to any considerable degree. Even in this case, the quantity usually present would not throw the result off more than a few per cent. Tragacanth, Indian gum, acacia, lactose, starch, and dextrin were tried. This would seem to show that the method may be carried out directly on salol in the tablet, without previous extraction with ether.

The reactions involved are the following:

First, the salol is saponified to sodium or potassium salicylate and phenolate:

$$HOC_6H_4CO.OC_6H_5+3KOH=KOC_6H_4CO.OK+C_6H_5OK+2H_2O$$

Phenol is attacked by bromin in excess to form symmetrical tribromophenolbromid:

$$C_6H_5OH+4Br_2=C_6H_2Br_3OBr+4HBr$$

Salicylic acid also forms the same product, inasmuch as the tribromosalicylic acid first formed is very unstable and loses its carboxyl:

$$C_6H_4OH.COOH+4Br_2=C_6H_2Br_3OBr+4HBr+CO_2$$

The tribromophenolbromid then reacts with hydriodic acid to give tribromophenol and free iodin:

$$C_6H_2Br_3OBr+2HI=C_6H_2Br_3OH+HBr+I_2$$

As a result, 12 atoms of bromid have been used up by 1 molecule of salol.

It is our intention to examine the various conditions affecting this method and determine the limits of its application. Although a large number of results have so far been obtained to establish the certainty that the method is applicable, they will not be published until the work is completed, when all the data will be collected and edited in one article.

REPORT ON MEDICATED SOFT DRINKS.

By Geo. W. Hoover, *Associate Referee.*

The work on medicated soft drinks is a continuation of the investigation of the methods presented to the association in 1910 by H. C. Fuller. The methods are outlined in Bulletin 137, page 190, and in Circular 66, page 7, with the following additional suggestions:

In view of the results of analysis obtained by the referee last year, it was recommended that this year approximately 10 cc of the sample be used for determination of solids in the place of 25 cc as indicated in the method submitted. In addition to this determination with 10 cc of the sample, I should be glad, if you find it convenient, to have a determination of the solids using approximately 2 grams for a sample. It was an oversight that solids were not determined at 100° C., instead of at 100° to 105° C.

The work was confined to the determination of the constituents caffein, cocain, phosphoric acid, and the estimation of the total solids. The cooperative sample was prepared in such a way as to essentially represent, in so far as possible, a large number of preparations of this character which were found upon the market a few years ago. Medicated soft drinks containing cocain are very seldom met with at present.

Cooperative Work on Medicated Soft Drinks.

CAFFEIN.

The amount of caffein used in the preparation of the sample is calculated to be 0.369 per cent. The results of the analyses of the majority of the chemists showed the product to contain from 0.36 to 0.37 per cent of caffein. One chemist reported 0.34, another 0.32, and still another 0.40 and 0.41 per cent. The principal criticism of the method by cooperators is that provision should be made for complete extraction of caffein in case the four chloroform shake-outs recommended are not sufficient. The figures show that the method gave concordant and accurate results for analysis of the sample in hand, but the above suggestion should be observed. Experience has shown that the caffein is obtained in quite pure state without subjecting it to a special method of purification, and it is believed that if the worker is careful in manipulation this constituent can ordinarily be determined accurately without purifying the product.

COCAIN.

The fluid extract of coca was used in the preparation of the sample, and it is estimated from the determination of the cocain in the fluid extract that the product should contain 0.0067 per cent of cocain. The results of the analyses of eight chemists showed the product to contain from 0.0045 to 0.006 per cent. One chemist reported 0.0029 and another 0.0038 per cent. The results all show slightly less cocain than the quantity calculated to be present. In view of the small quantity of the constituent, it is believed that the method outlined is satisfactory. The quantity of cocain that is usually found in preparations of the class under consideration is usually very small, and in view of its importance it is desirable that the method for its estimation be as accurate as possible.

PHOSPHORIC ACID.

The quantity of phosphoric acid used in the preparation of this sample is estimated to be 0.545 per cent. The results of analyses of eight chemists show the preparation to contain from 0.544 to 0.58 per cent. Two analysts obtained results as low as 0.52, but the majority of workers found from 0.544 to 0.570 per cent. The method employed requires three precipitations, and it is therefore necessarily long and more or less tedious. Several collaborators have suggested that the method should be shortened if possible, and further criticism is made that the quantity of the sample directed to be used in the determination is too large, for the reason that the precipitate formed is so heavy that it is found cumbersome to manipulate. The results as a whole are satisfactory, but it is deemed desirable to provide for the use of a smaller quantity of the material for sample, and that more work should be done with a view of shortening the method.

TOTAL SOLIDS.

The referee last year recommended that 10 grams of sample be used for the determination of the total solids in place of 25 cc, which was the recommendation of the original method presented to the association. It was also recommended that the residue should not be heated above 100° C. The results upon total solids show much variation, the majority ranging from 49.21 to 53.09 per cent. It was requested that total solids be determined, using a sample of from 2 to 3 grams on asbestos. The results obtained by using this comparatively small quantity for the sample indicate that a quantity of 2 to 3 grams is more satisfactory than 10 grams.

CONSTITUENTS AND QUANTITIES USED IN THE PREPARATION OF SAMPLE.

	Grams.
Water	8,984.07
Sugar	9,000.00
Phosphoric acid (sirupy)	170.55
Fluid extract of coca	251.47
Caramel	169.72
Alcohol	161.64
Lime juice [1]	100.03
Flavoring (oils of lemon, orange, anise)	21.78
Caffein	70.00
Total	18,929.26

	Per cent.
Caffein	0.369
Cocain	.0067
Phosphoric acid (P_2O_5)	.545

Comparative results on samples sent to collaborators.

Analyst.	Caffein.	Cocain.	Phosphoric acid (P_2O_5).	Solids.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Albrech, M. C., Pittsburgh, Pa.	0.37	0.005	0.58	43.61 (10 gram sample heated at 100° to 105° C. for 5 hours)
				45.52 (2 gram sample evaporated on steam bath and heated for 5 hours)
H. E. Buchbinder, Washington, D. C.	.364	.0038	.55	
E. O. Eaton, San Francisco, Cal.	.37	.006	.52	52.30 (10 gram sample)
				51.00 (3.5 gram sample)
H. C. Fuller, Washington, D. C.	.36	.0055	.56	53.09
G. W. Hoover, Washington, D. C.	[2] .363	.005	.58	52.82 (About 3 cc sample, 16 hours at 65° to 70° vacuum)
	[3] .367		.56	56.16 (About 10 cc sample, 16 hours at 65° to 70° vacuum)
				51.45 (About 2.5 cc sample, 20 hours at 65° to 70° vacuum)
				51.86 (About 2 cc sample, 10 hours at 65° to 70° vacuum)
				52.04 (About 2 cc sample, 10 hours at 65° to 70° vacuum)
				51.15 (About 2 cc on asbestos, 13 hours 65° to 70° vacuum)
				50.62 (About 3 cc on asbestos, 13 hours 65° to 70° vacuum)
				52.72 (About 2 cc sample, 13 hours at 65° to 70° vacuum)
				53.02 (About 2 cc sample, 13 hours at 65° to 70° vacuum)
C. H. Kimberly, Philadelphia, Pa.	[4] .34	.0055	.535	42.25 (About 10 gram sample, charred at 105° C.)
			.549	42.55 (About 10 gram sample, charred at 105° C.)
				38.28 (About 2 gram sample, charred at 105° C.)
				37.85 (About 1.7 gram sample, charred at 105° C.)
				51.80 (About 4.6 grams on asbestos, temperature below 100° C.)
				51.10 (About 2.6 grams on asbestos, temperature below 100° C.)
				53.09 (About 9.8 grams on asbestos, temperature below 100° C.)
				53.10 (About 10 grams on asbestos, temperature below 100° C.)

[1] Contains small amount of phosphoric acid.
[2] Not purified, 0.378 per cent; purified 0.363 per cent.
[3] Not purified, 0.374 per cent; purified, 0.367 per cent.
[4] Per cent calculated from the specific gravity.

Analyst.	Caffein.	Cocain.	Phosphoric acid (P_2O_5).	Solids.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
E. C. Merrill, Washington, D. C.	0.371	0.00546	0.587	59.61 (25 cc with sand vacuum oven, 65° to 70° C. for 5 hours) 58.20 (25 cc with sand vacuum oven, 65° to 70° C. for 5 hours) 52.08 (10 cc with Wiesnegg oven, 100° to 105° C. for 5 hours) 49.30 (5 cc with sand Wiesnegg oven, 100° to 105° C. for 5 hours)
		Gram per 100 cc.		
C. B. Morrison, New Haven, Conn.	.40 .41	0.0049	.56	
A. E. Paul, Chicago, Ill.	.37 .35	(4)	.57 .56	51.96 (10 cc sample) 49.66 (2 cc sample) 49.21 (2 cc sample on asbestos)
B. H. St. John, Washington, D. C.	.37	.0029	.521 .567	53.24 (About 25 cc on sand 100° to 105° C.) 52.56 (About 25 cc on sand 100° to 105° C.) 51.09 (About 25 cc on sand in vacuum, 65° to 70° C.)
		Per cent.		
H. L. Schulz, Detroit, Mich.	.326	0.0045	.544	45.10

[4] Not weighed. Ethyl benzoate test positive.

COMMENTS OF ANALYSTS.

M. C. Albrech: Regards the determination of total solids by means of the vacuum oven as the accurate method. The method for the determination of phosphoric acid is probably accurate, but cumbersome and requires too much time, that is, dissolving the magnesia precipitate with 10 per cent nitric acid and washing as directed requires much time, owing to the fact that the precipitate seems to be contaminated. A better method for the determination of phosphoric acid is to render alkaline with lime water, ignite, take up in nitric acid, and determine directly by adding ammonium molybdate in the usual manner. It is suggested that the quantity of sample directed for phosphoric acid determination is too large.

H. E. Buchbinder: Suggests that the solution should be tested with Wagner's reagent after four shake-outs with chloroform to determine whether or not the caffein has been completely extracted.

H. C. Fuller: Phosphoric acid: This method as it stands is the result of experience with about 200 assays and is about as short as it can be. The problem is a little peculiar. The mixture contains a large quantity of sugar; to oxidize this with nitric acid and sulphuric acid much time is required, danger of loss by frothing, and the disagreeable features attendant with fumes, etc. It is necessary to precipitate with molybdate in any case. I found that the quickest and surest way to get the phosphate away from the sugar was to use magnesia mixture. This first precipitate can not be ignited and weighed as magnesium pyrophosphate, as there is often calcium phosphate present and that comes out too; but by dissolving in acid and throwing out with molybdate the phosphate is free from calcium; then the phosphate-molybdate can be titrated or dissolved in ammonium hydroxid and precipitated as the magnesium salt. I do not see how the work can be done more rapidly and still get accurate results.

Total solids: I would not go much below 10 cc for the sample unless it is recommended that the quantity be weighed, for there is danger of introducing an error in trying to measure a small amount of a sirupy liquid. This error, of course, decreases as the volume of sample decreases.

Caffein: Use five shake-outs with chloroform instead of the original four recommended.

C. H. Kimberly: In the determination of solids not over 5 grams of sample should be used. Should not be heated above 100° C., and a vacuum oven is preferable for the determination of solids. In the determination of caffein, more shake-outs with chloroform should be used than are directed, in order to insure complete exhaustion. Sample should be weighed for determination when possible.

E. C. Merrill: Sample of 3 to 4 grams should be used with sand for determination of solids where solids are above 30 per cent.

C. B. Morrison: Cocain residue crystallizes with some foreign matter. Gave positive reactions to tests outlined for identification of cocain.

A. E. Paul: Two cubic centimeters for sample instead of 10 cc is a decided advantage for the determination of solids. The use of asbestos is unnecessary, making it convenient to determine solids and ash in the same sample.

H. L. Schulz: A smaller quantity of the sample should be used for the determination of phosphoric acid. It is difficult to wash the precipitate obtained from 25 cc free from acid, and less alkali is required to dissolve the precipitate.

The results obtained by the methods outlined for the determination of caffein and cocain indicate that the methods as a whole are essentially satisfactory. It is deemed desirable to do more work upon the determination of phosphoric acid and total solids. It is also deemed desirable to take up methods for the determination of other constituents frequently found in this class of preparations. It is therefore suggested that this work be continued during the ensuing year.

Adjourned at 1 p. m. to convene at 1.30.

WEDNESDAY—AFTERNOON SESSION.

REPORT ON THE DETERMINATION OF CAMPHOR BY THE HYDROXYLAMIN METHOD.

By E. K. Nelson.

A sample of spirits of camphor was carefully prepared according to the Pharmacopœia, containing 10 grams of camphor to 100 cc of sample, and sent out for the determination of camphor by the following method:

Modified Walther Method.—Measure accurately 20 cc of spirits of camphor at 25° C. into a flask of 200 cc capacity, add 2 grams of sodium bicarbonate, and then accurately, from a burette, 35 cc of hydroxylamin solution. Connect the flask with a reflux condenser and heat on a water bath to gentle ebullition for 2 hours; cool to 25° and pour 12 cc of a mixture of equal parts of concentrated hydrochloric acid and water through the condenser tube, followed by 50 cc of water.

Transfer to a 500 cc flask, rinsing out condenser and flask thoroughly, make to volume and filter. Titrate an aliquot, 50 cc, as follows: Add 1 drop of Methyl Orange and neutralize the mineral acid with half normal sodium hydroxid, add phenolphthalein, and titrate to a faint pink with tenth-normal sodium hydroxid. A blank must be run, using the same amount of hydroxylamin solution and 20 cc of alcohol in place of the camphor solution. The difference between the titrations of the sample and the blank represents the hydroxylamin converted into camphor oxim.

Each cubic centimeter of tenth-normal sodium hydroxid is equivalent to 0.0152 gram of camphor.

Hydroxylamin solution: Dissolve 20 grams of hydroxylamin hydrochlorid in 30 cc of water. Then add 125 cc of aldehyde-free alcohol.

Precaution: A solution of hydroxylamin hydrochlorid will give a faint pink color with Methyl Orange, which, however, will fade but slowly on adding alkali.

In neutralizing the free mineral acid in presence of Methyl Orange, therefore, observe the following precaution: Titrate until the pink color has almost but not quite faded, or until the color matches that of a hydroxylamin solution to which Methyl Orange has been added. A point will be found at which the color change is sudden; a slight pink tint will, however, remain.

Twenty-three collaborators have reported their results, which are as follows:

Comparative results on camphor.

Analyst.	Camphor.	Analyst.	Camphor.
	Grams per 100 cc.		*Grams per 100 cc.*
M. C. Albrech	9.65	W. A. Pearson	8.89
			8.58
G. M. Bartlett	9.00		8.69
	9.16		
	9.12	Average	8.72
Average	9.09	Wyatt H. Randall	8.91
			8.74
Walter H. Blome	10.25		8.34
			8.37
A. W. Broomell	8.66	Average	8.59
	8.55		
	8.47	E. L. Redfern	9.18
			7.98
Average	8.56		8.34
V. Coblentz	9.30	Average	8.50
	9.35		
Average	9.32	H. L. Schulz	8.44
			8.61
L. Junius Desha	8.96		8.80
	8.91	Average	8.62
Average	8.94	E. R. Squibb and Co. Laboratory	9.46
			9.32
E. O. Eaton	10.25	Average	9.39
	10.15		
Average	10.20	B. H. St. John	9.38
			9.32
R. P. Fischelis	10.69	Average	9.35
H. C. Fuller	9.55	A. R. Todd	9.01
			8.97
L. D. Havenhill	8.23	Average	8.99
	8.43		
Average	8.33	Chas. E. Vanderkleed	8.49
			8.52
C. H. Kimberly	8.85		8.85
			8.85
H. M. Loomis	9.72	Average	8.68
J. P. Milliken	10.41		
A. G. Murray	9.50	Average of all results	9.02
		Average results of individual analysts	9.24
J. M. Francis	8.78		
	8.11	Difference between maximum and minimum result	2.71
	9.13		
Average	8.67		

Average of all results is 0.98 per cent less than camphor actually present.

COMMENTS OF ANALYSTS.

W. A. Pearson: It is my experience that this method will result about 1 per cent lower than it should.

H. C. Fuller: There seems to be a little camphor unconverted after the two hours.

A. G. Murray: The quantity of hydroxylamin used is greatly in excess of the quantity required. The amount of sodium bicarbonate used is insufficient to liberate all the hydroxylamin. I have obtained a mixture of the sample and free hydroxylamin in alcoholic solution in a pressure flask, titrating this solution after acidifying with standard hydrochloric acid and diluting largely.

Wyatt W. Randall: I have come to the conclusion that the camphor is not completely converted by two hours' boiling with hydroxylamin with the oxim.

L. Junius Desha: From the polarimetric analysis of the sample I am satisfied that the above results are about 10 per cent low. On three other samples I used the hydroxylamin method, the results being in every case 5 to 10 per cent low. I can only conclude that the conversion of camphor to camphor oxim was not complete.

DETERMINATION OF PEPSIN IN LIQUIDS.

By V. K. Chesnut.

Among recent methods advanced for the estimation of pepsin the Hâta[1] and the Jacoby-Solm[2] methods have recommended themselves as possessing basic elements desirable for developing into a method adapted for official use in connection with the enforcement of the food and drugs act. The Hâta method is based on the measurement of a given amount of an emulsion of egg albumen and the formation of a clear solution by the digestion of the albumen. It is very similar to the ricin method, which was selected for the cooperative assay because it was apparent that the protein could be more accurately measured than in the other case and especially because it has received general recognition as an excellent assay process.

It is a fact which has repeatedly been demonstrated in the Bureau of Chemistry that certain liquid galenical preparations of pepsin lose some of their activity on standing, especially at room temperature. This has often been found to be true even with dilute solutions of pepsin in tenth-normal hydrochloric acid, but the decomposition was found to be so very slow in the latter case that in making up the 0.4 per cent solution used for cooperative assay it was decided to use this solvent. In order, however, to protect the pepsin further against molds, the acid was previously saturated with chloroform. Samples of this solution were mailed together with a sample of standard pepsin and one of "Ricin Präparat" to the cooperating analysts on July 20. The procedure studied was as follows:

Cooperative Work on Pepsin.

DETERMINATION OF PEPSIN IN LIQUIDS.

Preparation of sample.—Add to 50 cc of the liquid under examination the requisite quantity of either fifth-normal hydrochloric acid or water to make the preparation of tenth-normal acid strength when diluted with tenth-normal hydrochloric acid to 90 cc. Preserve the sample in a refrigerator.

Preparation of reagents.—(1) Standard pepsin: Powder a good grade of U. S. P. pepsin and pass it through a No. 60 sieve; dry in vacuo over sulphuric acid, pass again through a sieve, and preserve in a stoppered bottle over sulphuric acid. The exact pepsin equivalent of the dry powder must be ascertained by the U. S. P. process, and this may be expressed in percentage based on the supposition that the U. S. P. product is 100 per cent pure.

(2) Pepsin solutions: Weigh off definite amounts of the standard pepsin from a weighing tube into the requisite quantity of tenth-normal hydrochloric acid to make—

(*a*) A 1 per cent solution.

(*b*) A 0.1 per cent solution.

These should be freshly prepared, since weak solutions of pepsin in tenth-normal hydrochloric acid suffer decomposition on standing.

(3) Add 1 cc of tenth-normal hydrochloric acid to 9 cc of the sample.

(*a*) Immerse a stoppered flask containing 45 cc of the sample and 5 cc of tenth-normal hydrochloric acid in boiling water for 15 minutes and filter.

(*b*) Immerse a stoppered test tube containing 18 cc of solution 3 in boiling water for 10 minutes and, after cooling, add 2 cc of Solution 2a, and filter if necessary.

(4) Ricin solution: The cheap commercial "Ricin Präparat nach Jacoby" manufactured in Germany is ground to a No. 60 powder, thoroughly mixed and dried, and then stored in a desiccator. Digest 1 gram of this powder for one hour at 37.5° C. in 100 cc of 5 per cent sodium chlorid solution, cool, and filter.

Method.—To each of 15 tubes first add from a burette 2 cc of the ricin solution and 0.5 cc of tenth-normal hydrochloric acid. Heat to 37.5° C. and then add the quantities of Solutions Nos. 2b, 3, 3a, and 3b indicated in the following table. Measure

[1] S. Hâta. Über die Bestimmung des Pepsins durch Aufhellung von trüben Eiereiweisslosungen. Biochem. Zts., 1909, *23:* 179–185.

[2] Eugen Solm. Ueber eine neue Methode der Quantitativen Pepsinbestimmung und ihre klinische Verwendung. Zts. Klin. Med., 1909, *64:* 159–167.

off Solution 3a first and then pour in the solutions to be tested as rapidly as possible from graduated pipettes, taking note of the total time consumed in the process and beginning first with tube No. 1 and then following in natural sequence.

Tube No.	Time of digestion.	Series I.		Series II.		Series III.	
		Solution 3 added.	Solution 3a added.	Solution 3a added.	Solution 3b added.	Tenth-normal hydrochloric acid added.	Solution 2b added.
	Minutes.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
1	.....	0.00	1.00	1.00	0.00	1.00	0.00
2	68	.25	.75	.75	.25	.75	.25
3	42	.50	.50	.50	.50	.50	.50
4	28	.75	.25	.25	.75	.25	.75
5	19	1.00	.00	.00	1.00	.00	1.00

If the solutions to be tested are not clear they should be filtered repeatedly through a hardened filter. If, however, it be found that they can not thus be clarified, check tubes for comparing the end digestion products should be made containing the varying amounts of the preparation made up with tenth-normal hydrochloric acid in place of ricin.

After the addition of the solutions to be tested, the test tubes are immersed in the 37.5° C. bath at once, preferably placed in corresponding order in a partitioned square or oblong wire rack, such as is used in bacteriological work; they are shaken and examined from time to time for one or two hours, or left overnight in the case of very weak solutions. The time of beginning the digestion and also the time in minutes of complete digestion for each tube should be noted (preferably with a stop watch) as indicated in the table.

If the rate of digestion is the same in each series, 3 contains exactly 0.1 per cent of pepsin, the amount present in the original solution being 0.2 per cent. If the rate is more rapid in I than in II or III it is stronger, the comparative strength being closely indicated by the time of action in the tubes containing less of the solution. If the rate of clearing is more rapid in III than in II, the solution contains some substance which interferes with the action of the pepsin, and this must be removed in some such way as by dialysis[1] or evaporation in vacuo or at a low temperature until, upon reexamination and further dilution or concentration, the rate of digestion is identical or nearly so in each series. One cubic centimeter of 2b represents 0.001 gram of pepsin.

Smaller quantities of pepsin may be determined in the same way by comparing with more dilute solutions of standard pepsin. As small a quantity as 0.00005 gram of U. S. P. pepsin can thus be readily detected by its nearly complete solvent action on the ricin precipitate inside of two hours, and 0.000005 gram shows marked action on the ricin inside of the same time. After four hours' digestion, the absence of any appreciable solvent action, as judged by occular inspection, indicates the absence of pepsin. The results should be expressed in per cent calculated on the basis of pepsin of U. S. P. quality being 100 per cent.

RESULTS OF COLLABORATIVE WORK.

This method was tested by ten analysts with the following results:

			Pepsin found.
July	22. V. K. Chesnut, Washington, D. C	per cent	0.38
	23. Courtney Conover, Pittsburgh, Pa	do	.3
	26. H. T. Graber, Detroit, Mich	do	.2
	30. C. L. Barthen, Detroit, Mich	do	.1
Aug.	9. E. O. Eaton, San Francisco, Cal	do	.18
	9. M. M. Becker, Philadelphia, Pa	do	.11

[1] Dialysis tubes are made by pouring collodion into test tubes or small Erlenmeyer flasks, allowing it to dry a little, adding more collodion, drying again 2 to 5 minutes or until the collodion does not adhere to the finger when touched, and then removing the film and the glass and plunging it at once into water. The films should be kept moist until used. The special advantage of these tubes is that they are eminently adapted for quantitative work.

			Pepsin found.
Aug.	9.	W. W. McAbee, Indianapolis, Ind.....per cent..	0.1
	15.	E. R. Lyman, Portland, Oreg.....do....	.1
	30.	V. K. Chesnut, Washington, D. C.....do....	[1].2
	30.	V. K. Chesnut, Washington, D. C.....do....	[2].1
Sept.	9.	J. P. Milliken, Brooklyn, N. Y.....do....	.1
	26.	A. Mohn, Brooklyn, N. Y.....do....	.1
		Original strength of the solution.....do....	.4

In the letter accompanying the directions sent it was suggested that, since no substance was present in the pepsin solution under examination which would materially interfere with the digestion, the method, in this instance, could be greatly simplified and shortened by diluting 10 cc of the solution up to 100 cc with tenth-normal hydrochloric acid and comparing its activity directly with that of a 0.01 per cent solution of pepsin. The method thus suggested was essentially the Jacoby-Solm procedure. This suggestion was generally overlooked by the analysts, so that there was a tendency to report results only to the first decimal place. More accurate results would undoubtedly have been reported had comparison been made with the 0.01 per cent solution as suggested, for not only would cognizance have been taken of the second decimal place, but the longer digestion would have given better opportunity for comparing the rate of digestion. Several analysts made this observation. General provision is made for this procedure in the last paragraphs of the method, but I am convinced that it should be provided for specifically.

COMMENTS OF ANALYSTS.

J. M. Francis: I do not consider this test as being worthy of serious consideration for several reasons. In the first place it is exceedingly long, tedious, and complicated, and in the second place the end point is not at all satisfactory, or conclusive, especially if the sample under examination happens to be a mixture or a dark-colored compound or happens to contain some interfering substance.

E. L. Maines: A method designed to replace the official method should not be based upon the U. S. P. assay, which is inaccurate. The method would not prove practical to the average pharmacist, but is a much quicker process and more adaptable to the chemist than is the U. S. P. method.

W. W. McAbee: The method is excellent and delicate.

M. M. Becker: In noting the end point, allowance should be made for a very faint turbidity.

W. A. Pearson: The method possesses very distinct advantages over others.

E. R. Lyman: The period for complete digestion was not so clearly defined as the tables would seem to indicate.

DISCUSSION.

Lack of experience in determining the end point of the digestion is, as several analysts pointed out, really a serious consideration in accepting the second decimal figure, especially in the case of very weak preparations, but the use of a black background greatly facilitates the observation. With strong solutions, there is little difficulty in getting a satisfactory end point. With solutions containing more than 0.1 per cent of pepsin, however, the comparative speed of reaction is altogether too great for accurate comparison.

Inspection of the results shows wide variation. One particularly interesting feature about them is that they seem to indicate a somewhat rapid decomposition of the pepsin during the first week of storage, due undoubtedly to some extent to the summer temperature to which they were subjected and probably to the action of the chloroform placed in the hydrochloric acid to conserve the pepsin against the action of molds. The highest percentage found was obtained at Washington in a sample kept

[1] Sample kept in cold storage. [2] Sample kept at room temperature.

in cold storage and analyzed three days after it was made up. The same sample kept continuously in cold storage yielded 0.2 per cent 40 days later, while another held at room temperature during the same time yielded but 0.1 per cent. Some of the discrepancies in the figures obtained are undoubtedly due, however, to lack of experience in determining the end point, and more especially in interpreting the significance of the small differences noted in the comparatively rapid action of the slightly diluted pepsin.

The method as it was suggested that it be carried out is essentially the Jacoby-Solm method, which is not complicated. The method permits the presence in the pepsin solution of large quantities of interfering substances, because, with most solutions, pepsin is present in sufficient quantities, more than 0.1 per cent, to allow of copious dilution with tenth-normal hydrochloric acid. In pepsin solutions containing an exceedingly small quantity of pepsin in the presence of a large quantity of interfering substances, and in preparations containing no pepsin at all, the complicated process contributed by this laboratory is absolutely necessary and appears to be well adapted to fortify evidence, especially in court cases. Turbid and dark-colored solutions do give trouble, but by making check experiments with the same solutions, after sterilizing and adding known quantities of pepsin, this difficulty can be largely overcome. This could be further supplemented if necessary by making nitrogen determinations to ascertain the extent to which the ricin has been dissolved. By using greater quantities of the digestive and more ricin the delicacy of the method could thus be very greatly enhanced. In working with turbid solutions it is well to remember that they can not always be filtered without suffering loss of strength.

At the present time there is but one official method for the assay of pepsin, that given by the U. S. P. The new method is not intended to displace this. The official method is of no value where interfering substances are present in the mixture, as is apt to be the case in many galenicals, nor is it of much value where only very small amounts of pepsin are in the compound. Some analysts have apparently relied upon it too much in ascertaining whether or not pepsin was entirely absent from a preparation, for they have reported pepsin as present on the strength of the observation that the quantity of albumen left undigested in the check bottle was slightly greater than it was in those containing the solution tested. This is open to the serious criticism that the volume of undigested albumen left in check experiments is not constant, one sample naturally often becoming more compact than another. The method gives but a vague impression also of the quantity of pepsin in cases where deterrents are present for the amount of albumen dissolved is often far greater in the bottles containing the smaller quantity of the enzyms. The old process is tedious even for the assay of U. S. P. pepsin, while with the new one in the absence of badly interfering substances very rapid work may be accurately accomplished. If one desires to ascertain quickly whether or not a solution is approximately of 1 or of 0.1 per cent strength, a minute's digestion will show it in the former, and about 10 minutes in the latter case.

In the absence, therefore, of any general method for the assay of pepsin it is believed that the one outlined above is admirably adapted to accurate and general rapid work, and is therefore worthy of much further investigation. It is moreover well adapted for solids, for pepsin is generally very easily extracted by dilute acids. It is true that the method is a little weak in being based upon U. S. P. assay, but it seemed desirable to connect it up with this standard. The difficulty may eventually be remedied by the adoption of egg albumen carefully dried in a vacuum at a low temperature as a standard protein, as in the Hâta method. Egg albumen would have a great advantage over ricin in that it is a common commodity easily procured in fresh condition, and is unlike ricin in being nonpoisonous. For the purpose of cooperative assay it is apparent that only solid preparations can be used. It is therefore planned during the ensuing year to continue along this line.

DETERMINATION OF NITROGLYCERIN IN MEDICINAL TABLETS.

By A. G. Murray.

Two samples of molded tablets made of nitroglycerin and milk sugar only were sent to collaborators. The following instructions were submitted:

Methods for the Determination of Nitroglycerin in Medicinal Tablets.

I. Preparation of the sample.

Crush 25 tablets under 10 cc of ether. A 25 cc cylindrical graduate makes a convenient container and a stout glass rod is used to crush the tablets. Rinse the rod with a little ether, allow the insoluble material to settle, and decant the solution into a 50 cc graduated flask. No special care need be taken to prevent a little insoluble material from going into the flask. Wash the residue repeatedly with 5 cc portions of ether and decant the washings into the flask until it has been filled to the mark. Insert the stopper and mix well.

II. Estimation by the modified Scoville method.

Place 20 cc of the ethereal solution in a carefully dried and tared 50 cc beaker. (A second aliquot of 10 cc may be used as a check.) Evaporate the solvent in a vacuum desiccator charged with sulphuric acid; apply the vacuum gradually so as to prevent ebullition; leave the beaker in the vacuum 30 minutes after the ether has evaporated; weigh and calculate ether extract per tablet. Treat the residue with 2 cc of phenoldisulphonic acid reagent, rotating the beaker in such a way that the reagent comes into contact with the entire inner surface. After 10 minutes add water and wash into a 100 cc flask. (If a check analysis as suggested was made wash this into a 50 cc flask.) Dilute to the mark and place 10 cc representing 1 tablet in a 100 cc flask, add about 50 cc of water and a few drops more of potassium hydroxid solution (20 per cent) than is required to neutralize the acid. (Do not use sodium hydroxid.) Dilute to the mark and compare the color with that produced by a standard nitrate solution similarly treated. Use any convenient colorimeter or Nessler tubes.

Reagents and standards.—Phenoldisulphonic acid reagent: Dissolve 25 grams of pure white phenol in 150 cc of concentrated sulphuric acid, add 75 cc of fuming sulphuric acid (13 per cent SO_3), stir well, and heat for two hours at about 100° C.

Standard solution: Dissolve 0.7217 gram of pure potassium nitrate in 1 liter of water. Evaporate 10 cc of this solution just to dryness on the steam bath. Cool and treat the residue with 2 cc of phenoldisulphonic acid reagent, observing the precautions noted above and using a glass rod if necessary to aid the solution of the residue. After 5 or 10 minutes dilute to 250 cc. Each cubic centimeter of this solution contains 0.004 mg of nitrogen. Add an excess of potassium hydroxid solution to an aliquot of this solution and dilute to 100 cc. It is advisable to prepare a standard of approximately the same color as the unknown. Nitroglycerin is 5.4 times nitrate nitrogen.

III. Estimation by the modified Hay method.

Place 5 cc of the ethereal solution in a 50 cc beaker, dilute with 5 or 10 cc of alcohol and add about 5 cc of 0.5 per cent alcoholic potassium hydroxid. Cover with a watch glass and allow to stand 10 minutes. Place on steam bath; allow to boil, remove the watch glass, and when most of the liquid is evaporated add about 25 cc of water and leave on steam bath until about half the liquid has evaporated or until the odor of alcohol can no longer be detected. Cool and dilute to 250 cc. Each cubic centimeter of this solution represents 0.01 of a tablet. Introduce an aliquot representing 0.02 to 0.04 mg of nitroglycerin into a 100 cc graduated flask, dilute with sufficient water to make the volume 90 to 95 cc, add 1 drop of concentrated hydrochloric acid, then 2 cc of sulphanilic acid solution and 2 cc of naphthylamin hydrochlorid solution. Complete the volume with water. Prepare at the same time and in the same way standards containing known amounts of sodium nitrite. Stopper the flasks and mix well. Compare the colors after 30 minutes. Nitroglycerin is calculated by multiplying nitrogen found by 8.

Reagents and standards.—Sulphanilic acid solution: Dissolve 1 gram in 100 cc of hot water.

Naphthylamin hydrochlorid solution: Under a hood boil 0.5 gram of the salt with 100 cc of water for 10 minutes, keeping the volume constant. Filter and keep in a glass-stoppered bottle.

Standard solution of sodium nitrite: To a cold solution of about 2 grams of sodium or potassium nitrite in 50 cc of water add a solution of silver nitrate as long as a precipitate appears. Decant the liquid and thoroughly wash the precipitate with cold water. Dissolve in boiling water. On cooling the silver nitrite is precipitated. Dry the crystals in the dark at the ordinary temperature (preferably in a vacuum). Weigh out 220 mg of the dry silver nitrite, dissolve in hot water, and decompose with a slight excess of sodium chlorid. When the solution becomes clear dilute to 1 liter. Dilute 5 cc of this solution to 1 liter. This second dilution is the standard to be used. It contains 0.0001 mg of nitrite nitrogen per cubic centimeter.

Only nitrite-free water should be used in the estimation by the modified Hay method.

Tabulated Results of Collaborative Work.

Twenty reports were received and tabulated as follows:

Comparative results on nitroglycerin tablets.

Analyst.	Sample 1.			Sample 2.			Colorimeter.
	Extract.	Scoville.	Hay.	Extract.	Scoville.	Hay.	
M. C. Albrech, Pittsburgh, Pa	0.35	0.26	0.32	0.82	0.68	0.80	Schreiner.
F. J. Austin, New York, N. Y	.36	.32	.34	.76	.76	.66	None.
W. C. Bartholomew, Indianapolis, Ind	[1].34	.33	.32	[1].83	.80	.80	None.
E. O. Eaton, San Francisco, Cal	.33	.30	.28	.70	.54	.63	Schreiner.
	.36	.36	.30	.76	.54	.66	
H. Englehardt and O. E. Winters, Baltimore, Md.	.35	.36	.40	.79	.69	.80	None.
H. C. Fuller, Washington, D. C	.40	.40	.20	.76	.80	.80	None.
	.35			.80			
E. G. Grab, Nashville, Tenn	.47	.26	.36	1.04	.73	.91	Hehner.
	.52			1.12			
Fritz Heidlberg, Philadelphia, Pa	1.21	.28	.29	2.75	.67	.71	Kruss.
	1.08	.29	.30	2.59	.67	.67	
	1.48	.30	.29	1.70			
	2.50			1.70			
	2.70						
	1.96						
A. M. Henry, Tallahassee, Fla	[2].55	.34	.34	[2].92	.72	.73	Schreiner.
	[2].74			[2]1.31			
W. D. McAbee, Indianapolis, Ind	.47	[3].54	.53	.95	[3]1.08	1.12	None.
H. McCausland, Chicago, Ill			.37			.77	Kennicott-Sargent.
			.37			.77	
J. P. Milliken, Philadelphia, Pa	.32	.38	.33	.80	.54	.53	None.
A. G. Murray, Washington, D. C	[4].35	.35	.36	[4].84	.84	.84	Duboscq.
	[4].38	.35	.38	[4].80	.78	.82	
W. A. Pearson, Philadelphia, Pa	.50	.36	.40	.94	.65	.80	None.
J. R. Rippetoe, New York, N. Y	.40	.32	.36	.78	.72	.78	Duboscq.
		[5].34	.37		[5].82	.78	
Ralph B. Roe, New Haven, Conn	.45	.34	.32	.80	.75	.80	Duboscq.
W. L. Scoville, Detroit, Mich	.42	.32	.36	.89	.64	.80	Schreiner.
	.47	.27	.38	.88	.68	.80	
	.44			.92			
A. E. Stevenson, Lawrence, Kans	.44	.38	.42	.79	.81	.86	None.
B. H. St. John, Washington, D. C	.46	.41	.36	.98	.83	.78	Duboscq.
G. O. Zahner and E. L. Maines, Brooklyn, N. Y.	.60	.64	.64	.96	1.08	.96	None.
Average		.35	.36		.74	.78	

[1] Ether was evaporated in a vacuum desiccator over sulphuric acid and nitroglycerin was then allowed to stand one-half hour under a pressure of less than 1 mm.

[2] Could not get concordant results on ether extract. Figures given are maximum and minimum results.

[3] Results much too low were obtained when the directions were followed. Results reported represent collaborator's modification, but owing to difference in shade reading was difficult.

[4] Ether extract stood overnight in desiccator.

[5] Results on residues obtained by evaporating ether extract at room temperature in 4-inch porcelain dishes.

Suggestions and Criticisms by Collaborators.

B. H. St. John suggests that the difficulty of preparing the standard nitrite solution due to the slow settling collodial silver chlorid may be overcome by adding to the solid silver nitrite an excess of sodium chlorid dissolved in a small quantity of water, 15 or 20 cc. After the reaction is complete, the solution may be diluted as desired.

A. M. Henry: Could not get concordant results on ether extract. Letting the ether extract stay in the vacuum desiccator longer does not help, as the one that I dried the longest gave the highest ether extract.

A. E. Stevenson: In the Scoville method there seems to be a difference in the shade of yellow between the sample and the known solution, so that it is difficult to make an exact comparison.

J. R. Rippetoe: Experienced no difficulty in reading. I believe modified Scoville method less liable to error in manipulation, and evaporation at room temperature more expedient and reliable.

Zehner and Maines: So far as these methods are concerned they do not appeal to me as being satisfactory. Any colorimetric test is unreliable, owing to the widely varying results obtained in comparing the colors by different workers.

H. C. Fuller: I think Scoville's method is more accurate when dealing with the small quantities.

W. C. Bartholomew: Unless great care is taken in extracting with ether, appreciable amounts of nitroglycerin will remain in the residue. Compressed tablets are much more difficult to extract than molded tablets. Unless a very low pressure is obtained in the desiccator the nitroglycerin will not be dry in half an hour. The modified Scoville method seems preferable to the modified Hay method, but duplicate determinations do not agree as closely as might be desired.

R. B. Roe: In our judgment the modified Scoville method is the more satisfactory, especially with the Duboscq colorimeter.

H. McCausland: We could not secure agreeing results at all with the modified Scoville method. We found the yellow color a very difficult shade to compare colorimetrically; in fact, we could not secure results agreeing within 5 cc on the colorimeter. The modified Hay method proved very constant, giving identical results each time.

Chas. E. Vanderkleed: Hay's method would show any nitrous acid or nitrites in addition to nitroglycerin that might be present. Moreover, the Hay method seems to be almost too sensitive as the actual amount of nitroglycerin under observation is only about 0.008 mg, while in the Scoville method about 0.2 mg is under observation. For these reasons we should prefer the Scoville method.

A. D. Thorburn did not collaborate on the official samples. He manufactured several special lots of nitroglycerin tablets which he analyzed by the proposed methods. The results were submitted to the referee, together with the following remarks: "The residue from the ether extract was washed into a separator with 10 or 15 cc of water and extracted with ether, added in successive portions of 25 cc, 15 cc, 10 cc, and 5 cc. The ethereal solution was evaporated and treated as directed in the method submitted. The residue yielded from 4 to 16 per cent of the quantity of nitroglycerin claimed to be present in the tablets. Results similar to this have been obtained by three other laboratories in Indianapolis."

Discussion.

The results indicate that the methods as outlined, while undoubtedly capable of improvement, are trustworthy in the hands of a careful and experienced analyst. The ether extract can not, of course, usually be regarded as nitroglycerin, but its estimation is desirable as furnishing a maximum limit for nitroglycerin.

The results given in the table indicate that the tendency is toward high results in the ether extract. The probable reason for this is that the drying is not complete in the time recommended. Nitroglycerin appears to retain water with considerable tenacity.

The question of the completeness of the extraction of nitroglycerin from tablets deserves careful attention. In the case of molded tablets, such as were sent out for cooperative work, the quantity of nitroglycerin retained in the residue is probably negligible, but in the case of some compressed tablets appreciable amounts of nitroglycerin are not extracted by the proposed method.

As to the relative merits of the Scoville and Hay methods, there is a difference of opinion among the collaborators. The averages of results by the Hay method are slightly higher than those by the Scoville method and the agreement is somewhat better. In a determination of this character, where the quantity of material is small, where variations in the reagents may affect the results, and where some of the standards themselves are likely to deteriorate, it is fortunate that there are two independent methods available. In the absence of a satisfactory identity test the application of two such methods serves fairly well as a substitute, as it is not likely that any other

substances or mixtures of substances could be obtained which would be soluble in ether, nonvolatile in vacuo at room temperature, capable of giving the nitrate reaction with phenoldisulphonic acid, and on saponification would yield about two-thirds of the nitrogen in the form of nitrite. It is, therefore, not contemplated recommending one of these methods for adoption to the exclusion of the other, but rather to improve them in any way possible and propose both for adoption.

A STUDY OF THE LEAD NUMBER OF ASAFETIDA AND ALLIED PRODUCTS.

By E. C. Merrill and H. A. Seil.

The following method for the determination of the lead number of asafetida has been devised with the purpose of giving, not necessarily an absolute value, but a constant which, for the sake of comparison with figures obtained similarly with other products, would furnish a means of determining whether the asafetida has been adulterated:

Extract any convenient quantity of asafetida with alcohol, filter, evaporate the filtrate to a pasty consistency, add cold water, mix, and allow the residue to subside, then decant the liquid. Wash the precipitate with water two or three times more by decantation, drain the residue carefully and dissolve in U. S. P. ether with gentle warming if necessary. Then transfer the ethereal solution to a separatory funnel, wash two or three times with water, transfer to a porcelain evaporating dish, drive off the ether on a steam bath, and transfer the resin to a suitable container for future use.

Transfer about 1 gram of the sample of resin to an accurately weighed beaker, dry for five hours at 110° C., cool in a desiccator, weigh, and calculate the amount of material actually present in the beaker. Dissolve the dry resin in the beaker in 80 per cent alcohol, transfer to a 100 cc graduated flask, add 25 cc of a 5 per cent solution of lead acetate in 80 per cent alcohol, and make up the volume to the mark with 80 per cent alcohol. Thoroughly shake the mixture, allow to stand overnight, filter a portion, expel the alcohol from an aliquot part, and determine the lead present as sulphate by adding 10 cc of sulphuric acid (1 to 1 by volume). Then warm the mixture, add 100 cc of alcohol (95 per cent), boil for 10 minutes, allow to stand for one-half hour, filter onto a tared Gooch crucible and dry the contents one hour at 100° C., cool, and weigh. Run a control test using the same amounts of lead acetate and other reagents. The weight of lead sulphate in the control minus the lead sulphate in the test sample gives the amount of combined lead. The combined lead is calculated as milligrams of metallic lead per gram of sample. Factor=0.6830 or logarithm 9.8344.

Below is given a table showing the results obtained by this method and the character of the ether-purified resins:

Comparison of various lead numbers.[1]

Product.	Lead No.	Character of resin.
Asafetida	222	Hard, dry, slight odor of asafetida when cold; amber color.
Galbanum	4	Soft and plastic, slight odor; wine color.
Ammoniacum	75	Dark brown, soft and plastic; characteristic odor.
Olibanum	None.	Hard, brittle, transparent, light amber color, resinous odor, similar to colophony.
Guaiac	171	Hard, dry, brittle, dark amber color; no odor.
Myrrh	7	Hard, brittle, transparent, maroon color, slight myrrh odor.
Colophony	142	Hard, brittle, transparent resin; pale amber color; no odor.
Bdellium	55	Semisolid, dark red; no odor.
Sandarac	251	Hard, brittle resin, light straw color; crystallizes from ether in long, parallel needles; no odor.
Mastic	34	Hard, brittle, pale straw color; no odor.
Gamboge	9	Hard, brittle, light straw color; faint odor.
Dragon's blood	0	Hard, brittle, deep maroon or blood color; no odor.
Euphobium	34	Hard, dry, yellowish red; slight odor.
Pepper "Asafetida."	82	Semisolid, maroon color; sharp, penetrating, disagreeable odor when heated.

[1] This table shows the lead number of asafetida with the exception of sandarac, which is not a likely adulterant, to be much higher than any of the other products.

A number of samples of asafetida from different importations were examined according to the above method, and have shown the following variations:

Sample A (New York), 222 (Merrill), 218 (Seil).

Sample B (New York), 218.

Sample C (New York), 237.

Sample D (Washington), 250.

Sample E (New York), 210.

The amount of variation in the lead number appears to run between 200 and 250, while all the other products thus far examined, with the exception of sandarac, run much lower. It is interesting to observe that the lead numbers of galbanum and ammoniacum are nearly 200 points below that of asafetida, while olibanum has no lead number whatsoever. The values obtained are not absolute, inasmuch as the drying at 110° for 5 hours is incomplete, yet this method may be easily checked by independent workers, and the ratio of increase in the lead number after prolonged drying for several days is not sufficient to justify such a procedure.

COOPERATIVE WORK ON MORPHIN.

By H. E. Buchbinder.

The following samples were submitted to the collaborators:

Powdered opium; paregoric, prepared from the same opium according to the directions of the United States Pharmacopœia; and a sirup preparation of morphin containing sugar (40 grams per 100 cc), alcohol (5 per cent by volume), glycerin (5 per cent by volume), essential oil (a mixture of anise, lemon, and orange oils) (0.1 per cent by volume), caramel (0.165 grams per 100 cc), morphin sulphate containing approximately 3 per cent of other alkaloids as impurities (0.040 per 100 cc), water, q. s.

The methods as studied the preceding year were somewhat modified and resubmitted to the collaborators in 1912 in the following form:

ESTIMATION OF MORPHIN IN OPIUM.

Place 1 gram of the dried and powdered sample in a suitable flask, add 100 cc of lime water (U. S. P.) from a pipette, stopper, and shake thoroughly every 10 minutes, or continuously on the mechanical shaker, during 2 hours. Allow to settle and filter rapidly through a dry fluted filter. Remove 50 cc of the clear filtrate and transfer to a separatory funnel (No. 1). Shake out 7 times with washed chloroform (chloroform washed with water), using 30 cc each time; collect the chloroform in a separatory funnel (No. 2). Shake out once more with chloroform, transfer this chloroform into another separatory funnel (No. 3), containing 15 cc of clear lime water. Shake up funnel No. 3, withdraw the chloroform, filter, if necessary, and evaporate to dryness. Moisten the residue with a few drops of dilute acid, then add one drop of Mayer's reagent. If there is a precipitate, repeat this process of shaking out the original solution with chloroform, washing the chloroform with lime water in funnel No. 3, evaporating, and testing as indicated above, until Mayer's reagent gives a negative test. Add the lime water in funnel No. 3 to the chloroform in funnel No. 2, shake thoroughly, reject the chloroform, wash the lime water with 30 cc of chloroform, again reject the chloroform, and add the lime water to the original solution in funnel No. 1. Add 1.5 to 2 cc of a 10 per cent solution of ammonium chlorid; or else neutralize closely with acid, then add ammonia by drops, using no more than 3 or 4 drops in excess. Shake out 7 times with a mixture of chloroform and alcohol (2 parts of chloroform to 1 part of alcohol by volume), using 30 cc each time and collecting the chloroform alcohol in a separatory funnel. Shake out once more with chloroform alcohol, take about 5 cc of the last shake out, evaporate to dryness, moisten with a few drops of dilute acid, and test with Mayer's reagent. If there is a precipitate, repeat extraction and subsequent testing on a portion until Mayer's reagent gives a negative test. Combine the chloroform alcohol shake outs and wash once or twice with 10 cc of water, then in turn wash the water twice with an equal volume of chloroform alcohol, the latter to be added to the main chloroform alcohol. Evaporate the chloroform alcohol to dryness (on the steam bath), dissolve the residue in 10 cc of warm neutral alcohol, add a convenient excess of fiftieth-normal sulphuric acid, evaporate most of the

alcohol, and titrate back with fiftieth-normal potassium or sodium hydroxid, using cochineal (U. S. P. 5 drops) or methyl red (0.2 per cent in alcoholic solution 3 drops), as indicator. If methyl red is used the alcohol need not be evaporated; instead, dilute with about 10 or 15 cc of water before titrating back with alkali. Subtract the number of cubic centimeters of alkali from the number of cubic centimeters of acid used. One cubic centimeter of acid corresponds to 6 mg of morphin.

ESTIMATION OF MORPHIN IN PAREGORIC.

Evaporate 100 cc on the water bath to a volume not exceeding 15 cc. Transfer to a separatory funnel, using no more than 2 or 3 cc of water at a time for the rinsings and no more than 15 cc in all. Shake out twice with 25 cc of ether, collect the ether, and wash with 5 cc of water. Reject the ether and add the wash water to the main aqueous portion. To the latter add 30 cc of lime water, mix thoroughly, filter into a separatory funnel (No. 1), and wash with several small portions of lime water, using no more than 20 cc in all.

Proceed as in the method given for opium, beginning with the fourth sentence: "Shake out 7 times with washed chloroform," etc.

ESTIMATION OF MORPHIN IN SIRUP PREPARATIONS.

Take a convenient volume to yield between 30 to 75 mg of morphin; make acid, then ammoniacal, and extract to complete exhaustion with a mixture of chloroform and alcohol, using larger proportions of alcohol than that given above, if necessary to clear emulsions. In all cases do not consider the morphin exhausted until an actual test with 5 cc of the last shake out, carried out as indicated above in the method for opium (Mayer's reagent), shows it to be free from alkaloid. Do not test before at least 7 shake outs (not including the last shake out on a portion of which the test is to be carried out) have been made. Evaporate (on the water bath) the combined chloroform alcohol to dryness. Take up in 30 cc of lime water, filter into a separatory funnel, rinse, and wash several times with small portions of lime water, using about 20 cc in all.

Proceed as in the method for opium, beginning with the fourth sentence: "Shake out 7 times with washed chloroform," etc.

Comparative Results on Morphins.

The results are tabulated below:

Determination of morphin in opium, paregoric, and sirup.

Chemist or firm.	Morphin in opium.		Morphin in paregoric.	Morphin in sirup.	Chemist or firm.	Morphin in opium.		Morphin in paregoric.	Morphin in sirup.
	By U. S. P. method.	By method submitted.				By U. S. P. method.	By method submitted.		
	P. ct.	*P. ct.*	*Mg per 100 cc.*	*Mg per 100 cc.*		*P. ct.*	*P. ct.*	*Mg per 100 cc.*	*Mg per 100 cc.*
Clark and McCausland	12.22	12.36 11.64 11.88 12.24	.....	.....	Eaton	12.0	.....	[1] 42	30.0
					Fuller	.....	12.9	48.0	34.5
					Kimberly	.....	11.76	[1] 41.4	28.8
					Kinsley	.....	12.72	52.5	29.4
Average	.....	12.01							
					Loomis	11.86	13.94 13.40	.....	.....
Albrech	.....	11.64	.....	23.0 25.0					
					Average	.....	13.67		
Average	.....	.....	.....	[1] 24.0					
					McGee	12.05	13.45	[1] 42.0	33.0
Brown	.....	[1] 9.9	53.5 53.8	25.6 33 34	Mead	11.91	13.03 12.9	.....	.....
Average	.....	.....	53.65	30.9	Average	.....	12.97		
Bartlett	.....	[1] 9.9	.....	.....	Orrick	12.02	13.8	49.7	28.9
Buchbinder	11.92	.....	51.3	32.5					
Desha	.....	.....	53 51.5	.....	Palkin	.....	13 2 13.44	50.5	31.2
Average	.....	.....	52.25		Average	.....	13.32		

[1] Not included in the average.

Determination of morphin in opium, paregoric, and sirup—Continued.

Chemist or firm.	Morphin in opium.		Morphin in paregoric.	Morphin in sirup.
	By U. S. P. method.	By method submitted.		
	P. ct.	*P. ct.*	*Mg per 100 cc.*	*Mg per 100 cc.*
Francis	11.54	[1] 9.0	{ 44.0 [1] 43.0	31.2 31.8
Average				31.5
Roe		{ 12.0 12.12	48.6 48.0	32.0 31.0
Average		12.06	48.3	31.5
Rosengarten	11.95	13.25	49.4	31.5
Schulz	11.4	{ 13.7 13.4 13.2	49.0 48.6	.....
Average		13.43	48.8	
Squibb	11.7	11.12	{ [1] 28.2 28.4	.....
Taylor	11.79	11.98	{ 48.4 48.6	28.8
Average			48.5	
Wichmann		{ 12.6 12.42	49.8 50.4	30.0 29.7
Average		12.51	50.2	29.9
Mohn	11.0	{ 12.0 12.0 12.0	48.0 51.0	30.0 30.0 30.0 36.0
Average		12.0	49.5	31.5
General average.	11.79	12.62	50.27	30.9

	Opium.		Paregoric.	Sirup.
	U. S. P. method.	Method submitted.		
Total number of analysts taking part	12	20	18	16
Number of analysts included in the average	12	17	13	15
Number of analysts not included in the average	0	3	5	1
Number of analysts within 3 per cent of the average	10	4	7	7
Number of analysts within 5 per cent of the average	11	7	12	9
Number of analysts within 10 per cent of the average	12	16	13	14

[1] Not included in the average.

Comments by Collaborators.

M. C. Albrech: The powdered opium required about 25 extractions with chloroform to remove all other alkaloids from the lime-water solution and required about 10 extractions with chloroform alcohol to remove the morphin.

H. E. Buchbinder: In case of powdered opium there are two difficulties in the method. In the first place, with the treatment directed it is not always possible to extract the morphin completely. This could be remedied by longer digestion. The second difficulty, however, is insuperable. It is often practically impossible to shake out the other alkaloids from the lime-water solution, requiring sometimes as many as 25 shake-outs for a negative test. Furthermore, experiments have indicated that a negative test in this case is not reliable, as toward the end very little is extracted, even in a series of 5 shake-outs; nevertheless, the sum total of a number of series is considerable, and when the morphin is completely extracted in the first operation, leads to abnormally high results. The foreign alkaloids that are left in the lime water after a negative test is obtained are subsequently extracted along with the morphin by the chloroform alcohol mixture, which for those alkaloids is a better solvent than chloroform alone.

L. J. Desha: In case of paregoric 9 and 8 portions of washed chloroform were used in the first stage.

H. C. Fuller: Suggests that before evaporating the final shake-out the solvent might be run through a plug of cotton in the stem of the separatory funnel.

C. H. Kimberly: Methyl Red indicator was used. The transition point is not sharp when organic matter is present.

H. M. Loomis: In case of powdered opium, 14 and 17 shake-outs, respectively, were necessary with the chloroform in the first extraction before a negative test for alkaloids was obtained. In the shake-out with the chloroform alcohol mixture the extraction was complete in 7 shake-outs. More stress should be laid on the complete solution of the chloroform alcohol residue before titration.

W. H. Orrick: Seven shake-outs were sufficient at all stages of the assays, but very much trouble was encountered on account of emulsions.

R. B. Roe: Seven shake-outs sufficed in each case. The end points in the case of opium and paregoric were very obscure. Obtained higher results (12.6 per cent) on the opium by a modification in which the opium was thoroughly triturated in a mortar before shaking up with lime water. This method it would seem should give higher results, as we were unable to get perfect contact between the opium and lime water by the simple shaking method suggested.

F. Rosengarten: Used 8 shake-outs with chloroform and 8 shake-outs with chloroform alcohol in both the powdered opium and paregoric. The shake-out method is impracticable for the purpose of assaying opium, for the following reasons: On account of the small quantity of opium used for the assay the method is not suitable for assaying gum opium. The method is slow and tedious, and it involves great loss of solvents. The morphin obtained contains more or less coloring matter, which makes it difficult to observe the end point closer than 0.2 cc of fiftieth-normal solution. As 0.1 cc fiftieth-normal acid corresponds to 0.12 per cent morphin, this error is appreciable. Methyl Red was used as indicator and was found most satisfactory.

G. B. Taylor: Eight extractions by washed chloroform still showed alkaloids; 14 more gave reactions for alkaloid with Mayer's reagent. In a duplicate determination 17 chloroform extractions were required to rid the material of alkaloids. For paregoric 12 shake-outs with chloroform still gave a trace of alkaloid. Methyl Red gives satisfactory results.

E. R. Squibb and Sons: Both for the opium and the paregoric more than seven shake-outs were necessary. Methyl Red was found to be very satisfactory.

H. J. Wichmann: The paregoric required 12 shake-outs in the first stage and 9 in the last. The powdered opium required 14 shake-outs in the first and 9 in the last stage. The method is rather costly in that so much chloroform has to be used.

McCausland: We find the method very tedious. Our somewhat erratic results we attribute to the great difficulty in extracting the opium completely. We do not believe that the method offers any material advantage over the U. S. P. method.

Mr. Buchbinder presented a paper also on the Determination of Morphin, which may be published later by the Department of Agriculture.

A COMPARISON OF VALUES OBTAINED FOR THE REFRACTIVE INDICES OF AQUEOUS SOLUTIONS OF ETHYL AND METHYL ALCOHOLS.

By B. H. St. John.

In the routine examination of pharmaceutical preparations in the Drug Division of the Bureau of Chemistry it is necessary to make a great many alcohol determinations. The usual specific gravity determination is checked with the immersion refractometer, thus obtaining also an indication of the presence of methyl alcohol.

In connection with this work a comparison has been made of the alcohol tables given in the literature for use with this instrument. The tables of B. Wagner [1] and Leach and Lythgoe [2] are familiar to most chemists. L. W. Andrews [3] gives a series of values from 70 to 100 per cent by weight of ethyl alcohol at 25°. The values obtained by M. H. Deville [4] in 1842 are interesting from a historical point of view at least. They were determined at 16°. Paul Drude [5] gives a table of refractive indices of methyl alcohol solutions at 17°.

[1] Tabellen zum Eintauch Refraktometer, 1907.
[2] J. Amer. Chem. Soc., 1905, *27:* 964.
[3] Ibid., 1908, *30:* 353.
[4] Ann. chim. phys., 1842, v. 3, ser. 5.
[5] Zts. Physik. Chem., 1897, *23:* 300.

Recently Doroshevski and Dvorzhanchik[1] have published tables for ethyl alcohol solutions at 17.5° and 24° and for methyl alcohol at 15° and 17.5° and from these experimental data calculated a set of temperature coefficients which make it possible to reduce the values of all these investigators to a common temperature for comparison. By means of their temperature coefficients the values of all have been reduced to 17.5°. The refractometer readings were given corresponding to percentages by weight of alcohol.

Wagner's table was reduced to per cent by weight from per cent by volume, the ethyl alcohol table by the use of the table given in Circular 19 of the Bureau of Standards, and the methyl alcohol table by the table of Klason and Noorlen.[2]

Obtaining and maintaining a definite temperature is always attended with much difficulty, especially such a low temperature as 17.5° or 20° in the summer time. The ideal condition would be to be able to make the determination of the refractive index at room temperature, whatever that may be, noting the temperature with an accurate thermometer and interpolating in a table calculated by means of a temperature coefficient to give percentages of alcohol corresponding to refractometer readings at every degree centigrade in the usual range of the temperature of the laboratory, for example, from 17.5° to 30°.

Such a table is being calculated from the data given by Doroshevski.

The finished part has been used to some extent to interpret determinations in the way mentioned, and the figures obtained for per cent alcohol agree quite closely with the values obtained from specific gravity determinations on the same distillate.

Ethyl alcohol (per cent by weight).	Immersion refractometer readings at 17.5° C.					Ethyl alcohol (per cent by weight).	Immersion refractometer readings at 17.5° C.				
	Deville (1842).	Wagner (1903).	Leach and Lythgoe (1905).	Doroshevski (1908).	Andrews (1908).		Deville (1842).	Wagner (1903).	Leach and Lythgoe (1905).	Doroshevski (1908).	Andrews (1908).
0	16.46	15.00	15.00	15.00	.....	35	.....	77.10	77.84	77.72	.....
1	.....	16.55	16.62	16.49	.....	36	.....	78.37	78.98	79.00	.....
2	.....	18.13	18.25	18.21	.....	37	.....	79.62	80.08	80.22	.....
3	.....	19.77	19.75	19.90	.....	38	.....	80.80	81.20	81.39	.....
4	.....	21.45	21.37	21.62	.....	39	.....	81.92	82.33	82.50	.....
5	.....	23.13	22.98	23.36	.....	40	83.44	83.06	83.34	83.53	.....
6	.....	24.90	24.79	25.13	.....	41	.....	84.15	84.47	84.53	.....
7	.....	26.66	26.61	26.95	.....	42	.....	85.19	85.49	85.53	.....
8	.....	28.50	28.53	28.76	.....	43	.....	86.19	86.43	86.53	.....
9	.....	30.20	30.36	30.63	.....	44	.....	87.15	87.45	87.53	.....
10	34.14	32.34	32.18	32.53	.....	45	.....	88.18	88.50	88.50	.....
11	.....	34.28	34.03	34.45	.....	46	.....	88.98	89.33	89.40	.....
12	.....	36.22	35.87	36.40	.....	47	.....	89.86	90.16	90.28	.....
13	.....	38.14	37.82	38.37	.....	48	.....	90.72	91.09	91.11	.....
14	.....	40.07	39.66	40.34	.....	49	.....	91.53	91.91	91.89	.....
15	.....	41.98	41.49	42.34	.....	50	90.34	92.29	92.74	92.64	.....
16	.....	43.90	43.52	44.35	.....	51	.....	93.06	93.56	93.37	.....
17	.....	45.81	45.57	46.40	.....	52	.....	93.77	94.30	94.09	.....
18	.....	47.76	47.59	48.40	.....	53	.....	94.45	94.90	94.77	.....
19	.....	49.69	49.63	50.40	.....	54	.....	95.12	95.53	95.40	.....
20	50.91	51.64	51.69	52.40	.....	55	93.56	95.74	96.14	96.00	.....
21	.....	53.60	53.65	54.37	.....	56	.....	96.33	96.65	96.58	.....
22	.....	55.61	55.61	56.32	.....	57	.....	96.90	97.25	97.14	.....
23	.....	57.52	57.68	58.27	.....	58	.....	97.44	97.75	97.71	.....
24	.....	59.38	59.66	60.22	.....	59	.....	97.96	98.28	98.26	.....
25	.....	61.24	61.62	62.14	.....	60	94.64	98.47	98.79	98.74	.....
26	.....	63.11	63.48	63.97	.....	61	.....	98.94	99.31	99.23	.....
27	.....	64.85	65.36	65.78	.....	62	.....	99.37	99.74	99.69	.....
28	.....	66.62	67.21	67.49	.....	63	.....	99.78	100.15	100.11	.....
29	.....	68.28	68.96	69.19	.....	64	.....	100.19	100.65	100.50	.....
30	70.37	69.92	70.83	70.74	.....	65	.....	100.57	100.96	100.89	.....
31	.....	71.47	72.28	72.25	.....	66	.....	100.93	101.38	101.26	.....
32	.....	72.97	73.63	73.72	.....	67	.....	101.25	101.81	101.60	.....
33	.....	74.42	75.08	75.11	.....	68	.....	101.54	102.13	101.92	.....
34	.....	75.79	76.41	76.42	.....	69	.....	101.83	102.45	102.23	.....

[1] J. Russ. Phys. Chem. Soc., 1908, *40:* 101; 1909, *41:* 951.
[2] Arkiv. för Kemi, Mineralogi och Geologi, 1909, *2:* No. 27.

Ethyl alcohol (per cent by weight).	Immersion refractometer readings at 17.5° C.					Ethyl alcohol (per cent by weight).	Immersion refractometer readings at 17.5° C.				
	De-ville (1842).	Wag-ner (1903).	Leach and Lyth-goe (1905).	Doro-shevski (1908).	An-drews (1908).		De-ville (1842).	Wag-ner (1903).	Leach and Lyth-goe (1905).	Doro-shevski (1908).	An-drews (1908).
70	99.63	102.11	102.77	102.51	103.78	86	.....	102.68	102.66	102.86	103.64
71	.....	102.36	102.99	102.74	.....	87	.....	102.38	102.37	102.63	103.37
72	.....	102.58	103.20	102.94	.....	88	.....	102.05	102.08	102.34	103.05
73	.....	102.78	103.42	103.11	.....	89	.....	101.66	101.79	102.00	102.69
74	.....	102.95	103.63	103.26	104.40	90	100.13	101.27	101.49	101.60	102.26
75	.....	103.10	103.83	103.34	104.49	91	.....	100.76	101.19	101.11	101.77
76	.....	103.23	103.82	103.40	104.55	92	.....	100.23	100.69	100.56	101.21
77	.....	103.32	103.72	103.46	104.61	93	.....	99.62	100.09	99.97	100.59
78	.....	103.40	103.72	103.49	104.65	94	.....	99.45	99.29	99.26	99.89
79	.....	103.43	103.62	103.51	104.68	95	.....	98.25	98.55	98.49	99.00
80	102.74	103.45	103.52	103.51	104.67	96	.....	97.40	97.74	97.60	98.09
81	.....	103.48	103.42	103.49	104.52	97	.....	96.47	96.84	96.61	97.14
82	.....	103.38	103.33	103.43	104.37	98	.....	95.45	[1] 95.84	95.58	96.12
83	.....	103.28	103.24	103.34	104.19	99	.....	94.38	94.84	94.51	95.00
84	.....	103.14	103.15	103.23	104.03	100	94.64	93.37	93.83	93.37	93.78
85	.....	102.94	102.96	103.06	103.86						

[1] Bulletin 107, Rev., p. 100. Tables of Leach and Lythgoe, at 98 per cent alcohol, give a reading 92.0. This is a misprint, should be 93.0.

The values of Wagner and Doroshevski are given at 17.5° C. Those of Deville, Leach and Lythgoe, and Andrews, which are given at 16°, 20°, and 25°, respectively, have been calculated to 17.5° with the temperature coefficients given by Doroshevski.

Methyl alcohol (per cent by weight).	Immersion refractometer readings at 17.5° C.				Methyl alcohol (per cent by weight).	Immersion refractometer readings at 17.5° C.			
	Drude (1897).	Wagner (1907).	Leach and Lythgoe (1905).	Doro-shevski (1909).		Drude (1897).	Wagner (1907).	Leach and Lythgoe (1905).	Doro-shevski (1909).
0	15.69	15.00	15.00	15.00	50	41.83	41.37	41.68	40.87
2	16.69	16.00	15.94	16.10	55	41.44	40.95	41.40	40.39
5	18.19	17.79	17.80	17.84	60	40.41	39.95	39.98	39.35
7	19.61	19.12	19.02	19.12	65	38.71	38.19	37.65	37.81
10	21.42	21.20	20.85	21.18	70	36.60	35.93	35.25	35.21
12	22.46	22.71	22.13	22.68	75	32.35	32.77	32.00	32.22
15	24.97	24.90	23.95	24.91	80	29.45	28.81	28.35	28.39
20	29.17	28.88	27.48	28.58	85	24.68	24.23	24.20	23.75
25	32.18	32.37	30.80	32.23	90	19.05	18.83	18.55	18.39
30	35.22	35.43	34.05	35.12	95	13.17	12.68	12.10	12.21
35	38.24	37.79	37.28	37.46	100	7.25	6.05	4.58	5.64
40	40.55	39.74	39.68	39.29					

The values of Wagner and Doroshevski are given at 17.5°; those of Drude and Leach and Lythgoe, at 17° and 20°, respectively, have been calculated to 17.5°, with the help of the temperature coefficients given by Doroshevski.

The following memorials to M. A. Scovell and H. A. Weber, who had died since the last meeting of the association, were presented by the committee appointed to draft such resolutions, and the resolutions were adopted:

MEMORIAL TO MELVILLE A. SCOVELL.

Whereas Providence has removed by death from membership in this association our honored former president and coworker, Melville Amasa Scovell, of Kentucky: Therefore be it

Resolved, That in the death of Prof. Scovell this association has lost a member whose interest, enthusiasm, and active cooperation were enlisted in the upbuilding and

development of the work of this body along the present lines of its usefulness, while during the early formative period of its history his wise counsels and earnest support contributed largely to the success of this organization.

In the death of Prof. Scovell the cause of scientific agriculture has also sustained a heavy loss, since for many years he has been active in promoting improved methods of agriculture and in conducting research work along lines tending toward the fullest development of agriculture as a science.

Resolved, further, That this association desires to place on record this expression of appreciation of the work of our deceased colleague and of sorrow at his untimely death, and therefore be it

Resolved, That these resolutions be spread upon the minutes of this convention and that a copy be transmitted to the family of the deceased.

B. B. Ross, *Chairman*.
C. H. Jones.
E. W. Magruder.

MEMORIAL TO HENRY A. WEBER.

Whereas Providence has removed from our midst Henry Adam Weber, of Ohio, who has been for years prominently identified with the work of this association; who, from its organization, has been a member of the Committee on Food Standards, on which he has done most valuable work, who has been one of the pioneers in food work and one of its most faithful and valuable promoters; and who at all times has taken a deep interest in the work of this association; and

Whereas by his untimely death this association, the scientific world, and the cause of pure food have lost a most able, devoted, and valuable worker and the members of this association a sincere and helpful friend; be it

Resolved, That the Association of Official Agricultural Chemists by this token express the highest appreciation of the work of our departed member and friend and the deepest sorrow over our irreparable loss; be it further

Resolved, That these resolutions be spread upon the minutes of this meeting and published in suitable form for distribution among our members and other food chemists, and that a copy be sent to Mrs. Weber.

B. B. Ross, *Chairman*.
C. H. Jones.
E. W. Magruder.

The committee appointed to draw up resolutions to be sent to the directors of the State experiment stations requesting cooperation in the association work did not have their report ready for this meeting. The circular letter will be sent to the secretary for distribution.

The association adjourned.

LIST OF ANALYSTS WHO HAVE OFFERED TO COLLABORATE DURING THE LAST TWO YEARS.

Phosphoric acid: E. L. Baker, R. N. Brackett, J. E. Breckenridge, G. S. Fraps, H. D. Haskins, W. J. Jones, jr., H. B. McDonnell, A. McGill, A. J. Patten, Paul Rudnick, F. N. Smalley, R. E. Stallings, R. C. Thompson, F. P. Veitch, F. W. Woll.

Nitrogen—Determination: E. L. Baker, A. W. Bosworth, R. N. Brackett, J. E. Breckenridge, E. Peck Greene, C. L. Hare, H. D. Haskins, C. H. Jones, W. J. Jones, jr., H. B. McDonnell, A. McGill, E. W. Magruder, A. J. Patten, Paul Rudnick, F. T. Shutt, F. N. Smalley, R. E. Stallings, J. P. Street, R. C. Thompson, F. W. Woll.

Separation of nitrogenous bodies—Meat proteins: A. D. Emmett, A. McGill, Paul Rudnick, W. B. Smith, H. L. White.

Separation of nitrogenous bodies—Milk and cheese: A. W. Bosworth, A. McGill, O. B. Winter.

Separation of nitrogenous bodies—Vegetable proteins: G. A. Olson, T. B. Osborne, H. L. White.

Potash—Determination: J. W. Ames, E. L. Baker, R. N. Brackett, J. E. Breckenridge, W. H. Frazier, H. D. Haskins, R. Hoagland, W. J. Jones, jr., E. W. Magruder, H. B. McDonnell, A. McGill, A. J. Patten, B. F. Robertson, Paul Rudnick, F. T. Shutt, F. N. Smalley, R. E. Stallings, R. C. Thompson, W. E. Tottingham, F. P. Veitch, F. W. Woll.

Potash—Availability: J. W. Ames, R. N. Brackett, W. H. Frazier, H. D. Haskins, R. Hoagland, W. J. Jones, jr., T. E. Keitt, E. W. Magruder, A. McGill, Paul Rudnick, F. T. Shutt, F. N. Smalley, R. E. Stallings, R. C. Thompson, E. E. Vanatta, F. P. Veitch, F. W. Woll.

Soils: G. S. Fraps, A. M. Peter, J. H. Pettit.

Soils—Nitrogenous compounds: J. W. Ames, W. H. Frazier, R. Hoagland, E. W. Magruder, A. McGill, A. M. Peter, J. K. Plummer.

Dairy products—General: E. M. Bailey, J. O. Halverson, A. McGill, L. I. Nurenberg, H. J. Patterson, J. P. Street, G. B. Taylor, A. S. Wells.

Foods and feeding stuffs: G. L. Bidwell, C. S. Cathcart, G. S. Fraps, J. O. Halverson, R. Hoagland, M. E. Jaffa, W. J. Jones, jr., H. B. McDonnell, A. McGill, H. J. Patterson, F. N. Smalley, R. E. Stallings, F. W. Woll.

Sugar: A. H. Bryan, W. E. Cross, W. F. Hillebrand, W. D. Horne, A. McGill, S. F. Sherwood, M. N. Straughn, A. S. Wells.

Tannin: C. B. Bacon, A. McGill, J. S. Rogers, F. P. Veitch.

Insecticides: S. D. Averitt, G. E. Colby, C. C. McDonnell, A. McGill, A. V. H. Mory, A. J. Patten, H. J. Patterson, A. M. Peter, R. C. Roark, F. T. Shutt, H. V. Tartar.

Inorganic plant constituents: J. W. Ames, B. E. Curry, A. McGill, W. H. McIntire, A. J. Patten, J. H. Pettit, J. P. Street, R. C. Thompson.

Water: E. Bartow, R. N. Brackett, G. E. Colby, A. M. Henry, W. F. Hillebrand, P. W. Holtzendorff, A. McGill, A. J. Patten, Paul Rudnick, W. W. Skinner, H. J. Watson.

Testing chemical reagents: W. F. Hillebrand, A. McGill, E. C. Merrill, H. J. Patterson, J. B. Rather.

Colors: R. W. Balcom, C. S. Brinton, A. M. Doyle, H. C. Fuller, P. W. Holtzendorff, J. Hortvet, C. F. Jablon, W. W. Karnan, H. M. Loomis, H. L. Lourie, W. J. McGee, A. McGill, W. E. Mathewson, W. B. D. Penniman, H. L. Schulz, W. B. Smith, J. P. Street, L. M. Tolman, A. L. Winton.

Saccharine products: D. B. Bisbee, A. H. Bryan, J. R. Chittick, H. C. Fuller, A. McGill, S. H. Ross, F. L. Shannon, S. F. Sherwood, M. N. Straughn.

Fruit products: G. E. Colby, A. M. Doyle, H. C. Fuller, H. C. Gore, A. M. Henry, H. M. Loomis, H. L. Lourie, W. J. McGee, A. McGill, W. B. D. Penniman, S. H. Ross, F. L. Shannon, R. E. Stallings, S. W. Wiley, A. L. Winton.

Wine: D. B. Bisbee, H. C. Fuller, B. G. Hartmann, E. J. Lea, H. M. Loomis, H. L. Lourie, A. McGill.

Beer: H. C. Fuller, E. G. Grab, R. W. Hilts, J. Hortvet, H. M. Loomis, W. J. McGee, A. McGill, J. G. Riley, S. H. Ross, H. Runkel, H. L. Schulz, W. F. Sudro, L. M. Tolman.

Distilled liquors: A. B. Adams, H. C. Fuller, W. F. Hillebrand, W. W. Karnan, J. O. LaBach, H. M. Loomis, A. McGill, W. F. Sudro, L. M. Tolman.

Vinegar: R. W. Balcom, W. A. Bender, H. C. Fuller, F. W. Liepsner, E. R. Lyman, W. J. McGee, A. McGill, E. W. Magruder, W. B. D. Penniman, S. H. Ross, H. L. Schulz, F. L. Shannon, R. E. Stallings, J. P. Street, L. M. Tolman, S. W. Wiley.

Flavoring extracts: D. B. Bisbee, C. S. Brinton, E. M. Chace, W. L. Dubois, H. C. Fuller, R. S. Hiltner, P. W. Holtzendorff, J. Hortvet, C. F. Jablonski, F. W. Liepsner, H. L. Lourie, A. McGill, E. W. Magruder, C. D. Mason, A. E. Paul, W. B. D. Penniman, Paul Rudnick, H. L. Schulz, F. L. Shannon, B. H. Smith, R. E. Stallings, H. J. Watson, H. J. Wichmann, S. W. Wiley, A. L. Winton, B. B. Wright.

Spices: H. E. Barnard, C. S. Brinton, W. L. Dubois, H. C. Fuller, R. W. Hilts, A. McGill, W. B. D. Penniman, H. H. Rusby, H. E. Sindall, L. M. Tolman, H. J. Watson, S. W. Wiley.

Baking powder: C. S. Brinton, H. C. Fuller, M. E. Jaffa, A. L. Knisely, A. McGill, W. B. D. Penniman, R. E. Stallings, L. M. Tolman, S. W. Wiley.

Meat and fish: H. C. Fuller, H. M. Loomis, A. McGill, Paul Rudnick, W. B. Smith.

Fats and oils: H. S. Bailey, C. S. Brinton, W. L. Dubois, H. C. Fuller, C. F. Jablonski, R. H. Kerr, A. McGill, W. B. D. Penniman, Paul Rudnick, F. N. Smalley, W. B. Smith, S. H. Wiley.

Dairy products—Food adulteration: W. Alexander, H. S. Bailey, H. C. Fuller, J. O. Halverson, R. W. Hilts, J. Hortvet, F. W. Liepsner, E. W. Magruder, A. McGill, A. E. Paul, F. L. Shannon, G. B. Taylor, A. S. Wells, A. L. Winton.

Cereal products: H. C. Fuller, B. R. Jacobs, J. A. LeClerc, F. W. Liepsner, A. McGill, W. B. D. Penniman, H. H. Rusby, H. L. White, A. L. Winton.

Vegetables: H. C. Fuller, A. McGill, E. W. Magruder, J. P. Street.

Cocoa and cocoa products: H. S. Bailey, C. S. Brinton, W. L. Dubois, H. C. Fuller, R. W. Hilts, H. C. Lythgoe, A. McGill, S. H. Ross, H. H. Rusby, H. L. Schulz, R. E. Stallings, L. M. Tolman.

Tea and coffee: J. M. Bartlett, A. M. Doyle, H. C. Fuller, G. W. Hoover, M. E. Jaffa, A. L. Knisely, A. McGill, A. G. Murray, L. M. Tolman.

Preservatives: H. E. Barnard, C. S. Brinton, A. M. Henry, R. W. Hilts, W. W. Karnan, F. W. Liepsner, A. McGill, A. F. Seeker, F. L. Shannon, F. T. Shutt, R. E. Stallings, B. B. Wright.

Water in foods: H. C. Lythgoe, W. J. McGee, A. McGill, S. H. Ross, S. F. Sherwood, B. B. Wright.

Organic and inorganic phosphorus in foods: E. B. Forbes, A. McGill, J. Stewart, H. L. White, A. L. Winton.

Heavy metals in foods: W. Alexander, C. S. Brinton, H. C. Fuller, A. M. Henry, F. W. Liepsner, H. M. Loomis, A. McGill, A. V. H. Mory, W. B. D. Penniman, Paul Rudnick, R. E. Stallings, L. M. Tolman, A. S. Wells, H. J. Wichmann, A. L. Winton, B. B. Wright.

Medicinal plants and drugs: R. W. Balcom, C. S. Brinton, W. O. Emery, H. C. Fuller, A. M. Henry, G. W. Hoover, L. F. Kebler, H. M. Loomis, A. McGill, E. A. Ruddiman, H. H. Rusby, B. H. St. John, H. A. Seil, J. P. Street, W. F. Sudro, H. J. Watson, H. L. White.

ADDRESSES OF COLLABORATORS NOT INCLUDED IN PAGES 5 TO 8.

W. Alexander, Food and Drug Inspection Laboratory, U. S. Appraiser's Stores, New York, N. Y.

J. W. Ames, Agricultural Experiment Station, Wooster, Ohio.

E. L. Baker, Agricultural Experiment Station, Geneva, N. Y.

R. W. Balcom, Food and Drug Inspection Laboratory, Customhouse, Nashville, Tenn.

W. A. Bender, Food and Drug Inspection Laboratory, U. S. Appraiser's Stores, New York, N. Y.

D. B. Bisbee, Food and Drug Inspection Laboratory, St. Louis, Mo.

A. W. Bosworth, Agricultural Experiment Station, Geneva, N. Y.
C. S. Brinton, Food and Drug Inspection Laboratory, Philadelphia, Pa.
C. S. Cathcart, New Brunswick, N. J.
G. E. Coleby, University of California, Berkeley, Cal.
W. E. Cross, Sugar Experiment Station, Audubon Park, New Orleans, La.
B. E. Curry, Durham, N. H.
A. M. Doyle, Bureau of Chemistry, Washington, D. C.
W. L. Dubois, Food and Drug Inspection Laboratory, Buffalo, N. Y.
A. D. Emmett, Agricultural Experiment Station, Urbana, Ill.
E. B. Forbes, Agricultural Experiment Station, Wooster, Ohio.
W. H. Frazier, Agricultural Experiment Station, St. Paul, Minn.
E. Peck Greene, Stock Food Analyst, Tallahassee, Fla.
J. O. Halverson, Columbia, Mo.
C. L. Hare, Agricultural Experiment Station, Auburn, Ala.
H. D. Haskins, Agricultural Experiment Station, Amherst, Mass.
A. M. Henry, Department of Agriculture, Tallahassee, Fla.
W. F. Hillebrand, Bureau of Standards, U. S. Department of Commerce, Washington, D. C.
R. W. Hilts, Food and Drug Inspection Laboratory, Seattle, Wash.
R. Hoagland, Agricultural Experiment Station, St. Paul, Minn.
P. W. Holtzendorff, Shelby County Courthouse, Memphis, Tenn.
W. D. Horne, National Sugar Refinery, Yonkers, N. Y.
C. F. Jablon, Food and Drug Inspection Laboratory, U. S. Appraiser's Stores, New York, N. Y.
M. E. Jaffa, University of California, Berkeley, Cal.
W. J. Jones, jr., Purdue Experiment Station, La Fayette, Ind.
W. W. Karnan, Food and Drug Inspection Laboratory, U. S. Appraiser's Stores, New York, N. Y.
T. E. Keitt, Clemson College, S. C.
A. L. Knisely, Food and Drug Inspection Laboratory, Portland, Oreg.
J. O. La Bach, Agricultural Experiment Station, Lexington, Ky.
E. J. Lea, University of California, Berkeley, Cal.
F. W. Liepsner, Food and Drug Inspection Laboratory, Kansas City, Mo.
H. M. Loomis, Food and Drug Inspection Laboratory, San Francisco, Cal.
H. L. Lourie, Food and Drug Inspection Laboratory, U. S. Appraiser's Stores, New York, N. Y.
E. R. Lyman, Food and Drug Inspection Laboratory, Portland, Oreg.
A. McGill, Department of Inland Revenue, 317 Queen Street, Ottawa, Canada.
L. I. Nurenberg, Boston, Mass.
G. A. Olson, Agricultural Experiment Station, Pullman, Wash.
T. B. Osborne, Agricultural Experiment Station, New Haven, Conn.
A. E. Paul, Food and Drug Inspection Laboratory, Chicago, Ill.
W. B. D. Penniman, 6 East Franklin Street, Baltimore, Md.
A. M. Peter, Agricultural Experiment Station, Lexington, Ky.
J. H. Pettit, Agricultural Experiment Station, Urbana, Ill.
B. F. Robertson, Clemson College, S. C.
J. S. Rogers, Bureau of Chemistry, Washington, D. C.
S. H. Ross, Food and Drug Inspection Laboratory, Omaha, Nebr.
E. A. Ruddiman, Nashville, Tenn.
H. H. Rusby, College of Pharmacy, New York, N. Y.
H. L. Schulz, Food and Drug Inspection Laboratory, Detroit, Mich.
A. F. Seeker, Food and Drug Inspection Laboratory, U. S. Appraiser's Stores, New York, N. Y.

H. A. Seil, Food and Drug Inspection Laboratory, U. S. Appraiser's Stores, New York, N. Y.
F. L. Shannon, Dairy and Food Department, Lansing, Mich.
F. T. Shutt, Experimental Farm, Ottawa, Canada.
F. N. Smalley, Southern Cotton Oil Co., Savannah, Ga.
J. Stewart, Agricultural Experiment Station, Logan, Utah.
M. N. Straughn, Bureau of Chemistry, Washington, D. C.
W. F. Sudro, Agricultural College, N. Dak.
W. E. Tottingham, Madison, Wis.
E. E. Vanatta, Agricultural Experiment Station, Columbia, Mo.
H. J. Watson, Newark, Del.
A. S. Wells, 253½ Washington Street, Portland, Oreg.
H. J. Wichman, Food and Drug Inspection Laboratory, Denver, Colo.
S. H. Wiley, 15 South Gay Street, Baltimore, Md.
O. B. Winter, Agricultural Experiment Station, Geneva, N. Y.
A. L. Winton, Food and Drug Inspection Laboratory, Chicago, Ill.
F. W. Woll, Agricultural Experiment Station, Madison, Wis.
B. B. Wright, Food and Drug Inspection Laboratory, U. S. Appraiser's Stores, New York, N. Y.

OFFICERS AND REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1912–13.

Honorary president.

H. W. Wiley, Washington, D. C.

President.

G. S. Fraps, College Station, Tex.

Vice president.

E. F. Ladd, Fargo, N. Dak.

Secretary.

W. D. Bigelow, Washington, D. C.

Additional members of the executive committee.

C. H. Jones, Burlington, Vt.
R. N. Brackett, Clemson College, S. C.

Referees.

Phosphoric acid: A. J. Patten, East Lansing, Mich.
Nitrogen:
Determination of nitrogen: C. L. Hare, Auburn, Ala.
Separation of nitrogenous bodies: A. D. Emmett, Urbana, Ill.
Potash: H. B. McDonnell, College Park, Md.
Soils: G. S. Fraps, College Station, Tex.
Dairy products: E. M. Bailey, New Haven, Conn.
Foods and feeding stuffs: W. J. Jones, jr., La Fayette, Ind.
Food adulterations: Julius Hortvet, St. Paul, Minn.
Sugar: W. E. Cross, Audubon Park, New Orleans, La.
Tannin: C. B. Bacon, Washington, D. C.
Insecticides: S. D. Averitt, Lexington, Ky.

Inorganic plant constituents: W. H. McIntire, Knoxville, Tenn.
Medicinal plants and drugs: H. A. Seil, U. S. Appraiser's Stores, New York, N. Y.
Water: W. W. Skinner, Washington, D. C.
Testing chemical reagents: J. B. Rather, College Station, Tex.

Associate referees.

Phosphoric acid: L. S. Walker, Amherst, Mass.
Nitrogen:
Determination of nitrogen: R. N. Brackett, Clemson College, S. C.
Separation of nitrogenous bodies—
Milk and cheese: O. B. Winter, Geneva, N. Y.
Vegetable proteins: T. B. Osborne, New Haven, Conn.
Meat proteins: A. D. Emmett, Urbana, Ill.
Potash:
Determination: B. F. Robertson, Clemson College, S. C.
Availability: E. E. Vanatta, Columbia, Mo.
Soils: J. H. Pettit, Urbana, Ill.
Nitrogenous compounds: J. K. Plummer, Raleigh, N. C.
Dairy products: L. I. Nurenberg, Boston, Mass.
Foods and feeding stuffs: G. L. Bidwell, Washington, D. C.
Food adulteration:
Colors: W. E. Mathewson, New York, N. Y.
Saccharine products: J. R. Chittick, Des Moines, Iowa.
Fruit products: H. C. Gore, Washington, D. C.
Wine: B. G. Hartmann, Washington, D. C.
Beer: J. G. Riley, Washington, D. C.
Distilled liquors: A. B. Adams, Washington, D. C.
Vinegar: E. H. Goodnow, Washington, D. C.
Flavoring extracts: A. E. Paul, 1607 Transportation Bldg., Chicago, Ill.
Spices: H. E. Barnard, Indianapolis, Ind.
Baking powder: Edmund Clark, Boston, Mass.
Meat and fish: W. B. Smith, Kansas City, Mo.
Fats and oils: R. H. Kerr, Washington, D. C.
Dairy products: Julius Hortvet, St Paul, Minn.
Cereal products: H. L. White, Agricultural College, N. Dak.
Vegetables: E. W. Magruder, Richmond, Va.
Cocoa and cocoa products: H. C. Lythgoe, Boston, Mass.
Tea and coffee: J. M. Bartlett, Orono, Me.
Preservatives: A. F. Seeker, New York, N. Y.
Water in foods: W. J. McGee, New Orleans, La.
Organic and inorganic phosphorus in foods: E. B. Forbes, Wooster, Ohio.
Heavy metals in foods: H. M. Loomis, U. S. Appraiser's Stores, San Francisco,Cal.
Sugar: M. N. Straughn, Washington, D. C.
Insecticides: R. C. Roark, Washington, D. C.
Medicinal plants and drugs:
Synthetic products: W. O. Emery, Washington, D. C.
Medicated soft drinks: G. W. Hoover, Washington, D. C.
Medicinal plants: E. A. Ruddiman, Nashville, Tenn., and Adolph Ziefle, Agricultural College, N. Dak.
Water: G. E. Colby, Berkeley, Cal.
Tannin: F. P. Veitch, Washington, D. C.
Inorganic plant constituents: B. E. Curry, Durham, N. H.
Testing chemical reagents: E. C. Merrill, Washington, D. C.

Standing Committees.

Food Standards.

William Frear, State College, Pa., chairman.
E. H. Jenkins, New Haven, Conn.
R. E. Doolittle, Washington, D. C.
B. B. Ross, Auburn, Ala.
H. E. Barnard, Indianapolis, Ind.

Recommendation of Referees and Revision of Methods.

(Figures in parentheses refer to year in which appointment expires.)

P. F. Trowbridge, chairman.

Subcommittee A: A. J. Patten (1918), W. W. Skinner (1916), *B. B. Ross (1914), chairman, Alabama Polytechnic Institute, Auburn, Ala.* (Nitrogen, potash, phosphoric acid, soils, inorganic plant constituents, insecticides, water.)

Subcommittee B: F. W. Woll (1918), P. F. Trowbridge (1916), *E. M. Chace (1914), chairman, Bureau of Chemistry, United States Department of Agriculture, Washington, D. C.* (Dairy products, foods and feeding stuffs, sugar, tannin, medicinal plants and drugs, water in foods, separation of organic and inorganic phosphorus in foods, separation of nitrogenous bodies.)

Subcommittee C: L. M. Tolman (1918), *H. E. Barnard (1916), chairman, Indianapolis, Ind.*, C. D. Howard (1914). (Food adulteration.)

Special Committees.

Editing Methods of Analysis (Bulletin 107, Revised).

J. K. Haywood, Washington, D. C., chairman.
W. A. Withers, Raleigh, N. C.
J. P. Street, New Haven, Conn.
A. F. Seeker, U. S. Appraiser's Stores, New York, N. Y.
G. W. Hoover, Washington, D. C.
B. L. Hartwell, Kingston, R. I.

Practicability of Organizing for Study of Vegetable Proteins.

L. L. Van Slyke, Geneva, N. Y., chairman.
J. S. Chamberlain, Amherst, Mass.
J. M. Bartlett, Orono, Me.

Availability of Phosphoric Acid in Basic Slag.

C. B. Williams, West Raleigh, N. C., chairman.
C. G. Hopkins, Urbana, Ill.
H. D. Haskins, Amherst, Mass.
John S. Burd, Berkeley, Cal.
B. L. Hartwell, Kingston, R. I.

CONSTITUTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.

(1) This association shall be known as the Association of Official Agricultural Chemists of North America. The objects of the association shall be (1) to secure uniformity and accuracy in the methods, results, and modes of statement of analysis of fertilizers, soils, cattle food, dairy products, human foods, medicinal plants, drugs, and other materials connected with agricultural industry; (2) to afford opportunity for the discussion of matters of interest to agricultural chemists.

(2) Analytical chemists connected with the United States Department of Agriculture, or with any State, provincial, or national agricultural experiment station or agricultural college, or with any State, provincial, or national institution or body in North America charged with official control of the materials named in section 1, shall alone be eligible to membership; and one such representative for each of these institutions or boards, when properly accredited, shall be entitled to enter motions or vote in the association. Only such chemists as are connected with institutions exercising official fertilizer control shall vote on questions involving methods of analyzing fertilizers or involving definitions, nomenclature, laws, or regulations relating to fertilizers. Only such chemists as are connected with institutions exercising official cattle-food control shall vote on questions involving methods of analyzing cattle foods or involving nomenclature, definitions, laws, or regulations relating to cattle foods. Only such chemists as are connected with institutions exercising official food or drug control shall vote on questions involving methods of analyzing food or drugs or involving nonrenclature, definitions, laws, or regulations relating to food or drugs. All persons eligible to membership shall become members ex officio and shall be allowed the privileges of membership at any meeting of the association after presenting proper credentials. All members of the association who lose their right to such membership by retiring from positions indicated as requisite for membership shall be entitled to become honorary members and to have all privileges of membership save the right to hold office and vote. All analytical chemists and others interested in the objects of the association may attend its meetings and take part in its discussions, but shall not be entitled to enter motions or vote.

(3) The officers of the association shall consist of a president, a vice president, and a secretary, who shall also act as treasurer, and these officers, together with two other members to be elected by the association, shall constitute the executive committee. When any officer ceases to be a member by reason of withdrawing from a department or board whose members are eligible to membership, his office shall be considered vacant, and a successor may be appointed by the executive committee, to continue in office till the annual meeting next following.

(4) There shall be appointed by the executive committee, at the regular annual meeting, from among the members of the association, a referee and such associate referees for each of the subjects to be considered by the association as that committee may deem appropriate. [Construed by resolution passed in 1911 to mean the outgoing executive committee; standing rule adopted that the committee consult with each referee in the appointment of associates.]

It shall be the duty of these referees to prepare and distribute samples and standard reagents to members of the association and others desiring the same, to furnish blanks for tabulating analyses, and to present at the annual meeting the results of work done, discussion thereof, and recommendations of methods to be followed.

(5) The special duties of the officers of the association shall be further defined, when necessary, by the executive committee.

(6) The annual meeting of this association shall be held at such place as shall be decided by the association, and at such time as shall be decided by the executive committee, and announced at least three months before the time of meeting.

(7) No changes shall be made in the methods of analysis used in official inspection, except by unanimous consent, until an opportunity shall have been given all official chemists having charge of the particular inspection affected to test the proposed changes.

(8) Special meetings shall be called by the executive committee when in its judgment it shall be necessary, or on the written request of five members; and at any meeting, regular or special, seven enrolled members entitled to vote shall constitute a quorum for the transaction of business.

(9) The executive committee will confer with the official boards represented with reference to the payment of expenses connected with the meetings and publication of the proceedings of the association.

(10) All proposed alterations or amendments to this constitution shall be referred to a select committee of three at a regular meeting, and after report from such committee may be adopted by the approval of two-thirds of the members present entitled to vote.

By-Laws.

(1) Any amendment to these by-laws or addition thereto may be proposed at a meeting of the association and shall be published in the Proceedings. It may then be adopted by a majority vote of the association at the next meeting.

(2) These by-laws or any portion of them may be suspended without previous notice by a unanimous vote of those present at any meeting of the association.

(3) There shall be a committee of nine members which shall be designated as the committee on recommendations of referees. The president shall appoint three members of this committee to serve six years, such appointments to be made every other year as the terms expire. The chairman of the committee shall be appointed by the president and shall divide the nine members into three subcommittees (A, B, and C), and shall assign to each subcommittee the reports and subjects it shall consider.

(4) Each referee shall forward to the chairman of the committee on recommendations at least three weeks before the meeting of the association his recommendations and a sufficient abstract of his report to enable the committee to act intelligently on the recommendations.

As soon as possible after the annual meeting of the association each retiring referee shall transmit a copy of his report and recommendations, together with a statement of the action taken by the association upon the same, to the referee for the next year. [1911.]

(5) A method shall not be adopted as provisional or a provisional method amended until such method or amendment has been reported by the appropriate referee and published in the Proceedings of the association.

(6) A method shall not be adopted as official or an official method amended until such method or amendment has been recommended as official for at least two years by the appropriate referee.

(7) Each college, experiment station, bureau, board, or other institution entitled to representation in the association shall contribute annually $2, and its representatives shall not be qualified to vote or hold office in the association unless such annual dues have been paid, but these shall not be cumulative.

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